Single-cell Ca\textsuperscript{2+} parameter inference reveals how transcriptional states inform dynamic cell responses

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Single-cell genomic technologies offer vast new resources with which to study cells, but their potential to inform parameter inference of cell dynamics has yet to be fully realized. Here we develop methods for Bayesian parameter inference with data that jointly measure gene expression and Ca\textsuperscript{2+} dynamics in single cells. We propose to share information between cells via transfer learning: for a sequence of cells, the posterior distribution of one cell is used to inform the prior distribution of the next. In application to intracellular Ca\textsuperscript{2+} signalling dynamics, we fit the parameters of a dynamical model for thousands of cells with variable single-cell responses. We show that transfer learning accelerates inference with sequences of cells regardless of how the cells are ordered. However, only by ordering cells based on their transcriptional similarity can we distinguish Ca\textsuperscript{2+} dynamic profiles and associated marker genes from the posterior distributions. Inference results reveal complex and competing sources of cell heterogeneity: parameter covariation can diverge between the intracellular and intercellular contexts. Overall, we discuss the extent to which single-cell parameter inference informed by transcriptional similarity can quantify relationships between gene expression states and signalling dynamics in single cells.

1. Introduction

Models in systems biology span systems from the scale of protein/DNA interactions to cellular, organ and whole organism phenotypes. Their assumptions and validity are assessed through their ability to describe biological observations, often accomplished by simulating models and fitting them to data [1–4]. Under the framework of Bayesian parameter inference and model selection, the available data are used along with prior knowledge to infer a posterior parameter distribution for the model [5]. The posterior distribution characterizes the most likely parameter values to give rise to the data as well as the uncertainty that we have regarding those parameters. Thus, parameter inference provides a map from the dynamic phenotypes that we observe in experiments to the parameters of a mathematical model.

Single-cell genomics technologies have revealed a wealth of information about the states of single cells that was not previously accessible [6]. This ought to assist with the characterization of dynamic phenotypes. However, it is much less clear how to draw maps between dynamic phenotypes of the cell and single-cell states as quantified via genomic measurements. The challenge in part lies in the combinatorial complexity: even if a small fraction of genes contain information regarding the phenotype of interest, say a few hundred, this is more than enough to characterize any feasible number of states of an arbitrarily complex dynamical process.

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This leads us to a central question: can the integration of single-cell gene expression data into a framework for parameter inference improve our understanding of the cellular phenotypes of interest? Here, various sources of transcriptional noise must be taken into account [7–9], which we propose to address by taking a global view and comparing cells first by their similarity across many genes, and, after inference, by their similarity in posterior parameter distributions. Our previously work provides the ideal data for this approach: we jointly measured dynamics and gene expression in the same single cells [10]. Here, we apply our new parameter inference framework to study Ca\textsuperscript{2+} signalling dynamics and signal transduction in response to adenosine triphosphate (ATP) in human mammary epithelial (MCF10A) cells.

Ca\textsuperscript{2+} signalling regulates a host of cellular responses in epithelial cells, from death and division to migration and molecular secretion, as well as collective behaviours, such as organogenesis and wound healing [11–13]. In response to ATP binding to purinergic receptors, a signalling cascade is initiated whereby phospholipase C (PLC) is activated and in turn hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2), producing inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). The endoplasmic reticulum (ER) responds to IP3 by the activation of Ca\textsuperscript{2+} channels: the subsequent release of calcium from the ER into the cytosol produces a spiked calcium response. To complete the cycle and return cytosolic calcium levels to steady state, the sarco/ER Ca\textsuperscript{2+}-ATPase (SERCA) channel pumps the Ca\textsuperscript{2+} from the cytosol back into the ER [14,15]. Ca\textsuperscript{2+} signalling is highly conserved, regulating cell phenotypic responses across mammals, fish and flies [12,16,17] as well as in prokaryotes [18]. Since the Ca\textsuperscript{2+} response to ATP occurs quickly in epithelial cells: on a time-scale that is almost certainly faster than gene transcription, we work under the assumption that the transcriptional state of the cell does not change in the duration of the experiment.

Our ability to measure gene expression in thousands of single cells has not only led to new discoveries but has also fundamentally changed how we identify and characterize cell states [19]. Technologies used to quantify gene expression in single cells include sequencing and fluorescent imaging. The latter permits the measurement of hundreds of genes in spatially resolved populations of single cells. Small molecule fluorescence in situ hybridization (smFISH) can be multiplexed to achieve this high resolution by protocols, such as MERFISH [20] and seqFISH [21]. Moreover, by coupling multiplexed smFISH with fluorescent imaging of Ca\textsuperscript{2+} dynamics using a GFP reporter in MCF10A cells, we are able to jointly capture the dynamic cell responses and the single-cell gene expression in the same single cells [10]. These data offer new potential to study the relationships between transcriptional states of cells and the dynamic phenotypes these may produce.

Models of gene regulatory networks and cellular signalling pathways described by ordinary differential equations (ODEs) capture the interactions between gene transcripts, proteins or other molecular species and their impact on cellular dynamics. Well-established dynamical systems theory offers a range of tools with which to analyse transient and equilibrium behaviour of ODE models [22]; it remains an open question whether or not it is appropriate to make equilibrium assumptions of living cells [23]. Constraining dynamic models of cellular/molecular processes with single-cell data via inference offers much potential to gain new insight into dynamics, albeit coming with many challenges, given, among other things, the complex sources of noise in these data and the lack of explicit temporal information in (snapshot) datasets gathered at one time point [24]. Parameter inference has provided insight into clonal relationships of single cells [25,26] and stem cell differentiation/cell state transitions [27,28]. Inference methods have also been applied to single-cell data for the discovery of new properties of single-cell oscillations [29,30] and cell–cell variability [31–33], as well as to study cell–cell communication [34]. New methods to infer the parameters of models of stochastic gene expression provide means to study single-cell dynamics in greater depth [35,36].

Here, we model Ca\textsuperscript{2+} dynamics via ODEs based on previous work [37,38]. We develop a parameter inference framework to fit Ca\textsuperscript{2+} response dynamics in many single cells. We perform inference of multiple cells sequentially, through the construction of ‘cell chains’. A cell chain is an ordering of cells, which can be random or directed by some measure, e.g. by similarity of gene expression or of Ca\textsuperscript{2+} dynamic response. Given a cell chain, we propose to infer the parameters of the Ca\textsuperscript{2+} ODE model in a single cell via a transfer of information from its cell predecessor in the chain. We achieve this by setting the prior of the current cell in the chain informed by the posterior of its predecessor. We will use this framework to assess the extent to which transcriptional cellular states inform dynamic cell responses.

In the next section, we present the model and the methods implemented for parameter inference using Hamiltonian Monte Carlo in Stan [39]. We go on to study the results of inference: we discover that priors informed by cell predecessors accelerate parameter inference, but that cell chains with randomly sampled predecessors perform as well as those with transcriptional similarity-informed predecessors. Analysis of hundreds of fitted single cells reveals that cell-intrinsic versus cell-extrinsic posterior parameter relationships can differ widely, indicative of fundamentally different sources of underlying variability. By perturbing the posterior distributions, we assess model parameter sensitivities in light of Ca\textsuperscript{2+} dynamics. We also find that variability in single-cell gene expression is associated with variability in posterior parameter distributions, both for individual gene-parameter pairs and globally, via principal component analysis. We go on to cluster cells by their posterior parameter distributions, and discover that only for gene expression-based cell chains are there clear relationships between gene expression states and dynamic cell phenotypes.

### 2. Material and methods

#### 2.1. A model of Ca\textsuperscript{2+} dynamics in response to ATP

We model Ca\textsuperscript{2+} signalling pathway responses in MCF10A human epithelial cells using nonlinear ODEs, as previously developed [37,38]. The model consists of four state variables: phospholipase C (PLC), inositol 1,4,5-trisphosphate (IP3), the fraction of IP3-activated receptor (h) and cytoplasmic Ca\textsuperscript{2+}. The four variables are associated with a system of four nonlinear ODEs describing the rates of change of the Ca\textsuperscript{2+} pathway species following ATP stimulation, to characterize dynamic responses in MCF10A cells. The equations are given by

\[
\frac{d[PLC]}{dt} = \Delta\text{ATP} \cdot e^{-K_{ATP}} - K_{off,ATP}[PLC],
\]  

(2.1)
Ideally, each cell would be visited exactly once; however, this amounts to finding a Hamiltonian path in G, an NP-complete problem. Therefore, as a heuristic solution we use a depth-first search (DFS), which can be completed in linear time. From the current node, we randomly select an unvisited neighbour node and set this as the next current node, recording it once visited (pre-order DFS). If the current node has no unvisited neighbours, it backtracks until a node with unvisited neighbours is found. When there is no unvisited node left, every node in the graph has been visited exactly once. Given cases where the similarity matrix is sparse (as we have here), the DFS generates a tree that is very close to a straight path.

2.4. Bayesian parameter inference with posterior-informed prior distributions

We seek to infer dynamic model parameters in single cells, informed by cell–cell similarity via the position of a cell in a cell chain. We use the Markov chain Monte Carlo (MCMC) implementation: Hamiltonian Monte Carlo (HMC) and the No-U-Turn Sampler (NUTS) in Stan [39,42]. HMC improves upon the efficiency of other MCMC algorithms by treating the sampling process as a physical system and employing conservational laws [43]. From an initial distribution, the algorithm proceeds through intermediate phase of sampling (warm-up) until (one hopes) convergence to the stationary distribution. During warm-up, NUTS adjusts the HMC hyperparameters automatically [42].

The prior distribution over parameters is a multivariate normal distribution, with dimensions $\theta_j$, $j=1,\ldots,m$, where $m$ is the number of parameters. This choice of prior makes it straightforward to pass information from the inferred posterior distribution of one cell to the next cell in line to be sampled, which will be described in §2.5. Let $f$ be a numerical solution of the ODE model, and $y_0$ be the initial condition. Then, in each single cell, the Ca$^{2+}$ response to ATP is generated by the following process:

$$
\theta_j \sim N(\mu_\theta, \sigma_\theta^2)
$$

$$
y(t) = f(y_0, t; \theta)
$$

$$
\sigma \sim \text{Cauchy}(0, 0.05)
$$

where we truncate the prior so that each $\theta_j$ is bounded by 0 from below. The Cauchy distribution is chosen to generate the noise for observed Ca$^{2+}$ response as it contains greater probability mass in its tails, thus encouraging NUTS to explore extreme values of the parameter space more frequently.

For the first cell in a chain, we use a relatively uninformative prior, the ‘Lemon’ prior (table 1), derived from parameter value estimates in previous work [37,38,40]. For the $i$th cell in a chain ($i > 1$), the prior distribution is constructed from the posterior distribution of the $(i-1)$th cell (§2.5). For each cell, NUTS is run for four independent chains with the same initialization. To simulate $\tilde{y}(t)$ during sampling, we use the implementation of fourth- and fifth-order Runge–Kutta in Stan [39]. For each output trajectory $y$, its error is the Euclidean distance between $y$ and data $y^*$ for all 171 data points

$$
e(y, y^*) := \left( \sum_{i=1}^{171} (y_i - y^*_i)^2 \right)^{1/2}.
$$

The error of a posterior sample for a cell is the mean error of trajectories simulated from all draws in the sample

$$
\epsilon_{\text{sample}} := \frac{1}{N} \sum_{j=1}^{N} \epsilon(y_j, y^*_j).
$$

2.2. Data collection and preprocessing

The data consist of a joint assay measuring Ca$^{2+}$ dynamics and gene expression via multiplexed error-robust fluorescence in situ hybridization (MERFISH) [20]. Ca$^{2+}$ dynamics in a total of 5128 human MCF10A cells are measured via imaging for 1000 s (ATP stimulation at 200 s) using a GCaMP5 biosensor. Immediately following this step, 336 genes are measured for each Ca$^{2+}$ trajectory after this time. After removing the data for the first 200 s and downsampling by a factor of 10; the trajectories are at or close to steady state by this time. After removing the data for the first 200 s and downsampling for the last 700 s, each processed trajectory consists of Ca$^{2+}$ response data on 171 time points ($t=200, 201, \ldots, 298, 299, 300, 310, 320, \ldots, 1000$). All numerical experiments in this work will evaluate Ca$^{2+}$ response on those same 171 time points. Single-cell gene expression data are collected using MERFISH after the Ca$^{2+}$ imaging as previously described [10,20].

2.3. Generating cell chains via cell–cell similarity

Cell–cell similarity is quantified via single-cell transcriptional states, i.e. by comparing $x^i_{m}$ and $x^j_{m}$, the expression of $m$ genes in cells $i$ and $j$. We obtain a symmetric cell–cell similarity matrix, $W$ from the log-transformed MERFISH expression data via optimization in SotSPSC [41]. entries $W_{ij}$ denote the similarity between cells $i$ and $j$. To create a chain of cells linked through their similarity in gene expression space, we:

1. Construct a graph $G=(V, E)$; each node is a cell and an edge is placed between two cells if they have a similarity score above zero.
2. For a choice of initial (root) cell, traverse $G$ and record the order of cells traversed.
Table 1. Definition and description of the ODE model parameters. Prior distributions are derived from [37,38,40].

| name            | description                                                                 | prior distribution | unit                          |
|-----------------|-----------------------------------------------------------------------------|--------------------|-------------------------------|
| ATP             | concentration of ATP that activates PLC                                      | \( \mathcal{N}(5, 4) \) | \( s^{-1} \)                  |
| \( K_{\text{ATP}} \) | ATP decay rate                                                              | \( \mathcal{N}(0.0083, 0.0025) \) | \( s^{-1} \)                  |
| \( K_{\text{IP3}} \) | PLC degradation rate                                                         | \( \mathcal{N}(1.25, 1) \) | \( s^{-1} \)                  |
| \( V_{\text{PLC}} \) | maximum velocity for IP3 generation                                          | \( \mathcal{N}(1, 1) \) | \( \mu M \cdot s^{-1} \)        |
| \( K_{\text{IP}3} \) | equilibrium constant for IP3 generation through PLC                          | \( \mathcal{N}(5, 0.01) \) | \( \mu M \)                    |
| \( K_{\text{IP3IP}3} \) | IP3 degradation rate                                                        | \( \mathcal{N}(1.25, 1) \) | \( s^{-1} \)                  |
| \( a \)         | time constant of IP3 channel                                                 | \( \mathcal{N}(1, 1) \) | \( s^{-1} \)                  |
| \( d_{\text{IP}3} \) | dissociation constant for IP3 channel calcium inhibiting subunit             | \( \mathcal{N}(0.4, 0.01) \) | \( \mu M \)                    |
| \( K_{c} \)     | dissociation constant for calcium buffer                                    | \( \mathcal{N}(10, 4) \) | \( \mu M \)                    |
| \( B_{s} \)     | concentration of calcium buffer                                              | \( \mathcal{N}(150, 25) \) | \( \mu M \)                    |
| \( d_{1} \)     | dissociation constant for IP3 channel IP3 activating subunit                | \( \mathcal{N}(0.13, 0.01) \) | \( \mu M \)                    |
| \( d_{0} \)     | dissociation constant for IP3 channel calcium activating subunit            | \( \mathcal{N}(0.0823, 0.01) \) | \( \mu M \)                    |
| \( \epsilon \)  | ER to cytosolic volume                                                      | \( \mathcal{N}(0.185, 0.01) \) | —                             |
| \( \eta_{1} \)  | IP3 channel permeability constant                                            | \( \mathcal{N}(575, 625) \) | \( s^{-1} \)                  |
| \( \eta_{2} \)  | ER leak permeability constant                                                | \( \mathcal{N}(5.2, 1) \) | \( s^{-1} \)                  |
| \( \eta_{1} \)  | Ca\(^{2+}\) pump permeability constant                                       | \( \mathcal{N}(45, 25) \) | \( s^{-1} \)                  |
| \( \sigma_{0} \) | concentration of free Ca\(^{2+}\) in the ER                                | \( \mathcal{N}(4, 1) \) | \( \mu M \)                    |
| \( k_{3} \)     | SERCA pump dissociation constant                                            | \( \mathcal{N}(0.4, 0.01) \) | \( \mu M \)                    |

where \( N \) is the sample size and \( y_{i} \) is the output trajectory from the \( i \)th draw in the sample.

Convergence of NUTS chains is evaluated using the \( \hat{R} \) statistic: the ratio of between-chain variance to within-chain variance [39,44]. A typical heuristic used is \( \hat{R} \) between 0.9 and 1.1, indicating that for this set of chains the stationary distributions reached for a given parameter are well mixed. There are two caveats on our use of \( \hat{R} \) in practice:

1. For our model, we observe that well-fit (i.e. not overfit) Ca\(^{2+}\) trajectories did not require \( \hat{R} \in (0.9, 1.1) \) for all parameters. Thus, we assess \( \hat{R} \) only for the log posterior, using a more tolerant upper bound of 4.0.
2. There are cases where one chain diverges but three of the four are well mixed. In such cases, we choose to retain the three well-mixed chains as a sufficiently successful run. Thus if \( R \) is above the threshold, before discarding the run, we compute \( \hat{R} \) for all three-wise combinations of chains, and retain the run if there exist three well-mixed chains.

2.5. Constructing and constraining prior distributions

We construct the prior distribution of the \( i \)th cell from the posterior of the \((i-1)\)th cell. The prior mean for each parameter \( \theta_{j} \) for the \( i \)th cell is set to \( \mu_{j}^{(i-1)} \), the posterior mean of \( \theta_{j} \) from the \((i-1)\)th cell. The variance of the prior for \( \theta_{j} \) is derived from \( \sigma_{j}^{(i-1)} \), the posterior variance of \( \theta_{j} \) from the \((i-1)\)th cell. To (a) sufficiently explore the parameter space, and (b) prevent instabilities (rapid growth or decline) in marginal parameter posterior values along the cell chain, we scale each \( \sigma_{j}^{(i-1)} \) by a factor of 1.5 and clip the scaled value to be between 0.001 and 5. The scaled and clipped value is then set as the prior variance for \( \theta_{j} \) for the \( i \)th cell.

2.6. Dimensionality reduction and sensitivity analyses

To compare posterior samples from different cells, we use principal component analysis (PCA). Posterior samples are projected onto a subspace by first choosing a cell (the focal cell) and normalizing the posterior samples from other cells against the focal cell, either by min–max or z-score normalization. Min–max normalization transforms a vector \( x \) to \( (x - \min)/(\max - \min) \), where \( \min \) is the minimum and \( \max \) the maximum of \( x \). z-score normalization transforms \( x \) to \( (x - \mu)/\sigma \), where \( \mu \) is the mean and \( \sigma \) the standard deviation of \( x \). Normalizing to the focal cell amounts to setting \( \min = \max \), \( \mu = \mu_{j} \), \( \sigma = \sigma_{j} \) to be the values corresponding to the focal cell for all cells normalized. We perform PCA (implemented by scikit-learn 0.24 [45]) on the normalized focal cell posterior samples and project them into the subspace spanned by the first two principal components. The normalized samples from all other cells are projected onto the PC1–PC2 subspace of the focal cell.

We develop methods for within-posterior sensitivity analysis to assess how perturbations of model parameters within the bounds of the posterior distribution affect Ca\(^{2+}\) responses. Given \( \theta_{j} \), the posterior distribution of a cell, each parameter \( \theta_{j} \) is perturbed to two extreme values: \( \theta_{j}^{(0.01)}, \) the 0.01-quantile of \( \theta_{j} \), and \( \theta_{j}^{(0.99)}, \) the 0.99-quantile of \( \theta_{j} \). Nine ‘evenly spaced’ samples are drawn from the posterior range of \( \theta_{j} \) for the parameter of interest, \( \theta_{j}; \) the \( k \)th draw corresponds to a sample \( \theta_{j,k} \) such that \( \theta_{j,k} = \theta_{j}^{(0.1k)} \), the 0.1k-quantile of \( \theta_{j} \). For each draw \( \theta_{j,k} \), we replace \( \theta_{j} \) by either \( \theta_{j}^{(0.01)} \) or \( \theta_{j}^{(0.99)} \) and then simulate a Ca\(^{2+}\) response. The mean Euclidean distances between trajectories simulated from the evenly spaced samples and the perturbed samples are used to quantify the sensitivity of each parameter perturbation.

2.7. Correlation analysis and cell clustering of MERFISH data

Correlations between single-cell gene expression values and posterior parameters from the Ca\(^{2+}\) pathway model are determined for variable genes. We calculate the z-scores of posterior means for each parameter of a cell sampled from a population, and remove that cell if any of its parameters has a posterior mean z-score smaller than −3.0 or greater than 3.0. PCA is performed
on log-normalized gene expression of remaining cells using scikit-learn 0.24 [45], which yields a loadings matrix $A$ such that $A_{ij}$ represents the ‘contribution’ of gene $i$ to component $j$. We designate gene $i$ as variable if $A_{ij}$ is ranked top 10 or bottom 10 in the $j$th column of $A$ for any $j \leq 10$. For each variable gene, we calculate the Pearson correlation between its log-normalized expression value and the posterior means of individual model parameters. Gene–parameter pairs are ranked by their absolute Pearson correlations and the top 30 are selected.

For each cell, the posterior means for each cell are computed from parameter distributions. We predicted Ca$^{2+}$ responses of test cells not in $A_i$, using the initial conditions of the test cells but parameters from elsewhere. Each test cell was simulated using parameters sampled from: the posterior of a fitted cell with similar gene expression to the test cell; the posterior of a random fitted cell; and reference values from literature (‘Lemon’ values; see table 1). To compare predicted Ca$^{2+}$ responses, we used the Euclidean distance between a predicted Ca$^{2+}$ trajectory and data to quantify the prediction error. We found that posteriors of similar cells and posteriors of random cells had equally good predictive power on test cells: in both cases better than using Lemon values for prediction (figure 1d). These results illustrate how constructing priors for cells using posterior information from other cells offers greater ability to capture the dynamics of a new cell not previously modelled.

3. Results

3.1. Single-cell priors informed by cell predecessors enable computationally efficient parameter inference

To study the dynamic Ca$^{2+}$ responses of cells to ATP stimulation, we fit the ODE model (equations (2.1)–(2.4)) to data in single cells using Bayesian parameter inference (figure 1a). Only those MCF10A cells classified as ‘responders’ to ATP were included—cells with very low overall responses (less than 1.8 Ca$^{2+}$ peak height) were filtered out. To assess whether cell chains improve inference, we performed parameter inference of the ODE model in single cells fit either individually, each from the same prior (we used the ‘Lemon’ prior (table 1)), or fit via the construction of a cell chain. In a cell chain there is a transfer of information, whereby the posterior parameter distribution of one cell informs the prior distribution of the next cell in the chain. The first cell in the chain was fit using the Lemon prior. We are primarily interested in cell chains constructed using transcriptional similarity: we constructed cell chains based on a single-cell gene expression similarity metric and compared them with alternatives (see Material and methods; electronic supplementary material, table S1). We studied the effects of different choices of $g$, where $\pi_{i+1} = g(p_i)$, $p_i$ is the posterior distribution for cell $i$, and $\pi_{i+1}$ is the prior distribution for the following cell. We found that transformations via scaling and clipping were necessary to sufficiently explore the parameter space for each cell while maintaining stable marginal posterior distributions along a cell chain (electronic supplementary material, section S1.1, figure S2). We tested various numerical methods to solve the ODE system (stiff and non-stiff), and found that we could simulate Ca$^{2+}$ responses sufficiently well using a non-stiff solver (electronic supplementary material, figure S3), so for inference runs with hundreds of single cells we proceeded to use a non-stiff solver.

Parameter inference of the ODE model via a cell chain (denoted Similar-r1) was more efficient and gave more accurate results than individually fit cells (figure 1b), with shorter overall computational run times and higher posterior model probabilities (electronic supplementary material, table S2). The model fit quality was also higher for the cell chain versus individually fit cells as assessed by the $R$ statistic (electronic supplementary material, table S3). To test whether these improved model fits are in part due to longer fitting times rather than the construction of the cell chain directly, we fit the same cell consecutively ten times: the fits improved over the 10 repeat epochs, but the only substantial improvements were seen for the first couple of epochs, after which improvements were minimal and the overall fit quality was comparable to the same cell fitted in the chain (electronic supplementary material, section S1.2, figure S4a–f), albeit with some evidence for overfitting in individual parameters (electronic supplementary material, figure S4g,h). Thus, the quality of fits obtained from fitting in a cell chain are not inherently due to more time spent running inference but are due to the transfer of information between different cells along the chain.

The advantage of transfer learning in cell chains can be demonstrated by the higher predictive power of sampled posterior distributions. We predicted Ca$^{2+}$ responses of test cells for which the parameters have not been inferred (i.e. cells not in Similar-r1), using the initial conditions of the test cells but parameters from elsewhere. Each test cell was simulated using parameters sampled from: the posterior of a fitted cell with similar gene expression to the test cell; the posterior of a random fitted cell; and reference values from literature (‘Lemon’ values; see table 1). To compare predicted Ca$^{2+}$ responses, we used the Euclidean distance between a predicted Ca$^{2+}$ trajectory and data to quantify the prediction error. We found that posteriors of similar cells and posteriors of random cells had equally good predictive power on test cells: in both cases better than using Lemon values for prediction (figure 1d). These results illustrate how constructing priors for cells using posterior information from other cells offers greater ability to capture the dynamics of a new cell not previously modelled.

To assess whether cell chains ordered using gene expression information improve inference performance over cell chains ordered randomly, we compared inference runs of at least 500 cells in a chain, with priors informed by cell predecessor, where the chain construction was either random or gene expression similarity based. The performance of cell chains ordered randomly—evaluated by computational efficiency (sampling times) and accuracy of fits (model posterior probabilities)—was not significantly different to that of the similarity-based chains (electronic supplementary material, table S4). Therefore, although the use of a cell chain (priors informed by cell predecessors) improved inference relative to individually fit cells, the choice of cell predecessors (similarity-based versus randomly assigned) did not affect computational efficiency or the accuracy of fits.

We also studied the effects of HMC parameters on sampling. We found that sampling times were faster without loss of fit quality when we reduced the maximum tree depth (a parameter controlling the size of the search space) from 15 to 10, since rarely was a tree depth greater than 10 used in
practice; so this reduction did not negatively impact the model fits (electronic supplementary material, table S5). We also found that a warm-up period of 500 steps was sufficient for convergence of MCMC chains for most cells. Setting the maximum tree depth to 10 and the number of warm-up steps to 500 led to much faster sampling times for large populations of cells (figure 1e).

3.2. Analysis of single-cell posteriors reveals divergent intracellular and intercellular sources of variability

The posterior distributions of hundreds of cells show striking differences between marginal parameters: some are consistent across cells in a chain while others vary widely. To quantitatively assess this, we ran two similarity-based cell chains with identical cell ordering for the final 100 cells but with different initial cells. We found that while some marginal posterior parameters were similar for all cells (e.g. \(K_{\text{off}}\), ATP, figure 2a), others diverged for the same set of cells along a chain (e.g. \(d_5\), figure 2b). Relative changes in marginal posteriors were seen to be tightly correlated. We computed the fold change in mean marginal posterior parameter values between consecutive cells along the chain (figure 2a,b, second row): the majority of consecutive cell pairs were tightly correlated both in direction and magnitude, even when the absolute values diverged. We obtained similar

Figure 1. Cell chains improve performance of single-cell parameter inference. (a) Workflow for single-cell parameter inference along a cell chain. \(\pi_i\) and \(p_i\), respectively, denote the prior and posterior distributions of cell \(i\). MAP: maximum a posteriori value. (b–c) Comparison of inference for gene expression similarity-based cell chain (Similar-r1; first column) versus cells fit individually, i.e. without a cell chain (second two columns). Output metrics are the mean log posterior, where higher values denote better fits; and the sampling times. Each row represents a cell. For Similar-r1, one cell was sampled from a Lemon prior before Cell 5121 to inform the prior of that cell (not shown). Run 1 for the individually fitted cells has 500 warm-up steps and run 2 has 1000. (d) Comparison of predictions of the dynamics of an unfitted cell using: samples from the posterior distribution of a fitted cell with similar gene expression, samples from the posterior distribution of a random fitted cell, and reference values from literature ('Lemon' values; see table 1). (e) Comparison of the effects of HMC parameters. Parameters (num. warmup steps; max. tree depth) are for r1: (500, 10), r2: (1000, 15), r3: (500, 15).
results for random cell chains run in parallel with different initial cells (electronic supplementary material, figure S5).

Analysis of the posterior values of parameters relative to their ‘Lemon’ values (see table 1) revealed in some cases large distances between them (e.g. $d_5$ varies from 0.08 (Lemon) to posterior values greater than 3). There are several possible causes for these prior–posterior discrepancies, including differences in the biological system and differences in experimental inputs, e.g. the stimulus used or the amount of stimulus that cells receive.

Further analysis of the marginal posterior distributions revealed two uninformative (‘sloppy’ [49]) parameters. The posterior distributions of $B_i$ and $\eta_1$ drifted, i.e. varied along the chain independent of the particular cell (electronic supplementary material, figure S6a,b). Given these insensitivities, we studied model variants where either one or both of these parameters are fixed to their posterior mode values.

Figure 2. Parameter dependencies revealed by the analysis of marginal posterior distributions along cell chains. (a) Marginal parameter posterior distributions for the PLC degradation rate ($K_{off,ATP}$) in two parallel runs of the same similarity-based cell chain. Box plots of the first–third quartiles of the distribution with whiskers denoting its full range (upper). Marginal posterior mean fold changes of consecutive cells in the chain (lower). (b) As for (a), with the IP3 channel dissociation parameter ($d_5$). (c) Left: intercellular variability. Scatter plot of the maximum a posteriori (MAP) values of 500 cells for parameters $\eta_3$ and $c_0$. Colour indicates position along the chain. Right: intracellular variability. Scatter plots of 500 samples from the posterior of one cell from the chain; three representative cells are shown. (d) As for (c), with parameters $\epsilon$ and $\eta_2$. (e) As for (c), with parameters $K_{off,ATP}$ and $K_{ATP}$. 
parameters were set to a constant. Comparing chains of 500 cells each, the reduced models performed as well as the original in terms of sampling efficiency and convergence (electronic supplementary material, figure Snc-e and table S6). Posterior predictive checks of the reduced models showed no significant differences in simulated Ca$^{2+}$ trajectories. Thus, for further investigation into the parameters underlying single-cell Ca$^{2+}$ dynamics, we analysed the model with both $B_i$ and $\eta_j$ set to a constant. This cell chain is referred to as Reduced-3.

We discovered striking differences between intracellular and intercellular variability through analysis of the joint posterior distributions of parameters in chain Reduced-3. Several parameter pairs were highly correlated, as can be expected given their roles in the Ca$^{2+}$ pathway, e.g. as activators or inhibitors of the same species. However, comparison of parameter correlations within (intra) and between (inter) cells yielded stark differences. Some parameter pairs showed consistent directions of correlation intercellularly (along the chain) and within single cells. The Ca$^{2+}$ pump permeability ($\eta_d$) and the concentration of free Ca$^{2+}$ ($c_e$) were positively correlated both inter- and intra-cellularly (figure 2c). Similarly, the ER-to-cytosolic volume ($\epsilon$) and the ER permeability ($\eta_m$) were negatively correlated in both cases (figure 2d). However, the ATP decay rate ($K_{\text{ATP}}$) and the PLC degradation rate ($K_{\text{cal,ATP}}$) were positively correlated along the chain (posterior means) but—for many cells—negatively correlated within the cell (figure 2e). The distribution of MAP values was well-mixed, i.e. there is no evidence of biases arising due to a cell’s position in the chain: the variation observed in the posterior distributions represents biological differences in the population. These differences may be in part explained by the differences in scale: intercellular parameter ranges are necessarily as large as (and sometimes many times larger than) intracellular ranges. On these different scales, parameters can be positively correlated over the large scale but negatively correlated locally, or vice versa. These divergent sources of variability at the inter- and intra-cellular levels highlight the complexity of the dynamics arising from a relatively simple model of Ca$^{2+}$ pathway activation.

### 3.3. Quantifying the sensitivity of Ca$^{2+}$ responses in a population of heterogeneous single cells

We conducted analysis of the sensitivity of Ca$^{2+}$ responses to the model parameters. Typically, one defines a parameter sensitivity as the derivative of state variables with respect to that parameter [50,51]. Here, we are most interested in how the dynamics are affected by parameter perturbations over the range of their marginal posterior distributions. Thus, we evaluate the model response to a given parameter perturbation across its marginal posterior distribution in a population of cells as follows. First, sample from a cell’s posterior distribution, and alter each sample such that the parameter of interest is set to an extreme value according to its marginal posterior distribution (0.01-quantile or 0.99-quantile). We then simulate trajectories from these altered samples (figure 3d), and use the distance between unperturbed and perturbed model trajectories to define the sensitivity of model output to that parameter, taking the mean of nine simulated trajectories.

We find that there is a lot of variation in the Ca$^{2+}$ responses: sensitive to some model parameters and insensitive to others (figure 3b). Notably, the sensitivities of the least sensitive parameters had mean values of close to 1.0: similar to the distances obtained from the best-fit posterior values (electronic supplementary material, table S5), i.e. the Ca$^{2+}$ response is insensitive to these parameters across the whole posterior range. The insensitive parameters were not simply those which had the highest posterior variance: there was little correlation between the inferred sensitivity and the posterior variance (electronic supplementary material, table S7), compare e.g. parameters $d_i$ and $d_3$.

Analysis of the Ca$^{2+}$ responses to parameter perturbations provides means to predict how much Ca$^{2+}$ responses are affected by changes in extracellular and intracellular dynamics (figure 3c,d). For example, low concentrations of ATP result in very low Ca$^{2+}$ responses; increasing the concentration of ATP can more than double the peak response (figure 3c). The importance of IP3 in Ca$^{2+}$ signal transduction is in agreement with the results of Yao et al. [38]; here we go further in that we can quantify the particular properties of the Ca$^{2+}$ response affected by each parameter. In the case of $K_{\text{cal,IP3}}$, the main effect is also in the peak height of the Ca$^{2+}$ response (figure 3d).

### 3.4. Variability in gene expression is associated with variability in Ca$^{2+}$ dynamics

We studied variation between pairs of genes and parameters sampled from a cell population to assess whether relationships between them might exist. We found that several gene-parameter pairs were correlated. In general, the proportion of variance explained between a gene–parameter pair was low; this is to be expected given the many sources of variability in both the single-cell gene expression and the Ca$^{2+}$ responses.

Analysis of the most highly correlated gene–parameter pairs (see Material and methods and electronic supplementary material, table S8) identified a number of genes that were correlated with multiple parameters, e.g. PPP1CC, as well as parameters that were correlated with multiple genes, e.g. $\eta_3$. Pairwise relationships were analysed via linear regression. The top four correlated gene–parameter pairs from a similarity-based cell chain are shown in figure 4a–d: cells are well mixed according to their positions along the chain, i.e. correlations are not due to local effects. The pairwise correlations overall are low, which we expect given single-gene inputs. Performing multiple regression could improve predictive power; however, our goal here is to study whether any evidence supports the existence of individual gene–parameter relationships. We performed the same analysis on a randomly ordered cell chain, where the same gene–parameter relationships were recapitulated, albeit with lower absolute correlation values (figure 4e–h and electronic supplementary material, table S9). There is no discernable influence of a cell’s position in a chain on the gene–parameter relationship, confirming that these correlations among a cell population reflect the variability in the population rather than any sampling artefacts.

We compared the top genes ranked by gene–parameter correlations for four populations: from two randomly sampled and two similarity-informed cell chains. Gene–parameter pairs were sorted by their absolute Pearson correlation coefficients, and the genes ranked by their positions among sorted pairs. In total, we identified 75 correlated gene–parameter pairs for
These results are also suggestive of how information content encapsulated in the parameter posterior distributions. Reduced-3 the Sensitivity of Ca$^{2+}$ responses to parameter perturbations. \( \theta \) to baseline and perturbed trajectories. \( \theta \) sensitivities for a population of fitted cells from a gene expression similarity-based chain sampled across the posterior range. The parameter to be perturbed quarters of the cell chains studied (figure 4 of the top 30 genes, 20 also appeared as top-10 marker genes from unsupervised clustering (into three clusters) of the gene expression genes/parameters to that of the whole: what is the relation-ship between the posterior parameter distribution of a cell and its global transcriptional state? We used PCA for dimen-sionality reduction of the posterior distributions to address this question. We selected a cell (denoted the focal cell) from a similarity-based cell chain. We projected its posterior distribution using PCA. We projected the posterior distributions of other cells onto the first two components of the focal cell (figure 4jk and electronic sup-plementary material, figure S8) to evaluate the overall similarity between the posterior distributions of cells relative to the focal cell. On PCA projection plots, posterior samples

Next, we turn our attention from the level of individual genes/parameters to that of the whole: what is the relationship between the posterior parameter distribution of a cell and its global transcriptional state? We used PCA for dimensionality reduction of the posterior distributions to address this question. We selected a cell (denoted the ‘focal cell’) from a similarity-based cell chain (Reduced-3 model). Sensitivities are reported in terms of model distances between baseline and perturbed trajectories. Simulated model trajectories in response to perturbations in ATP concentration. Simulated model trajectories in response to perturbations in K$_{off,IP3}$. The high degree of overlap between these gene sets demonstrates that a subset of genes expressed in MCF10A cells explain not only their overall transcriptional variability but also their variability in Ca$^{2+}$ model dynamics. These results are also suggestive of how information content pertaining to the heterogeneous Ca$^{2+}$ cellular responses is encapsulated in the parameter posterior distributions.
are coloured based on gene expression: samples are derived from cells that are either transcriptionally similar to the focal cell, or share no transcriptional similarity. Comparison of similar and dissimilar cells from the same population showed that cells that were transcriptionally similar were located closer to the focal cell than dissimilar cells (figure 4l,m and electronic supplementary material, figure S8c,d). By contrast, similar analysis of a random cell chain showed that transcriptionally similar cells were not located closer to the focal cell than dissimilar cells (electronic supplementary material, figure S9). Notably, proximity of posterior samples derived from transcriptionally similar

Figure 4. Variability in Ca\textsuperscript{2+} model dynamics is associated with variability in gene expression. (a–d) Top ranked gene–parameter pairs by Pearson correlation from similarity-based chain (Reduced-3). Log gene expression is plotted against marginal posterior means. Each dot represents a cell; colour denotes cell position along the chain. (e–h) The same gene–parameter pairs shown in (a), for cells sampled from a randomly ordered cell chain (Random-2). (i) Comparison of genes identified as present/absent in the top 30 genes from gene–parameter correlation analysis across different chains, and the top 30 marker genes from Leiden clustering directly on the gene expression space. (j) Projection of posterior samples (500 per cell) onto the first two principal components of the focal cell (Cell 5106), shown in blue. Posterior samples from dissimilar cells shown in grey; posterior samples from similar cells shown in yellow-red, where the shade indicates degree of transcriptional similarity. (k) As for (j), with PCA performed on a different focal cell (Cell 4940). (l) Histogram corresponding to (j): mean distances from the origin (focal cell 5106) to samples from surrounding cells. (m) Histogram corresponding to (k): mean distances from the origin (focal cell 4940) to samples from surrounding cells.
Late-high-responders were characterized by small values of parameters leading to faster and earlier IP3 and PLC dynamics. Early responders exhibited earlier overall Ca2+ peaks in response to ATP; and non-responding cells, unlike Yao’s early responders. In our results, smaller in the late-high-responder cluster, but not in the figures S11 provided. That is, parameter inference of single-cell Ca2+ dynamics from using a gene expression similarity-based chain enables the identification of cell clusters with distinct transcriptional profiles and distinct responses to ATP stimulation.

We analysed differential gene expression from each set of clusters obtained, from the similarity-based chain (figure 5c), the gene expression-based clustering (figure 5f) and the randomly ordered chain (figure 5g). Distinct markers for each cluster were observed for the similarity-based clustering and the gene expression-based clustering, but were not discernible for cell clustering on the random chain. Clustering of cell posteriors from the randomly ordered chain was thus unable to distinguish Ca2+ dynamic profiles nor gene expression differences. On the other hand, clustering posteriors from a similarity-based chain identified distinct gene expression profiles, and these overlapped with the marker gene profiles obtained by clustering on the gene expression directly. That is, parameter inference of single-cell Ca2+ dynamics from using a gene expression similarity-based chain enables the identification of cell clusters with distinct transcriptional profiles and distinct responses to ATP stimulation.

Analysis of the genes that are associated with each Ca2+ profile showed that low-responder cells were characterized by upregulation of CCDC47 and PPI family genes (PPP1CC and PPP2CA). Early-responder cells were characterized by upregulation of CAPN1 and CHP1, among others. The late-high-responder cells were characterized by increased expression CALM3 among others, although the marker genes for this cluster were less evident than the others. By comparison of marker genes, we saw considerable overlap between the early-responders (similarity-based clustering) and the Ca-mid responders (gene expression clustering). We also saw overlap between the low-responder and the Ca-low marker gene signatures. These results highlight that the posterior distributions of cells fit from similarity-based cell chains captured information about the underlying transcriptional states of the cells, linking cellular response parameters directly to transcriptional states. For example, the low-responder cells (similarity-based clustering) were distinguished by parameter $d_s$, characterizing the dynamics of IP3 dissociation, and were marked by high PPP1CC and CCDC47 expression.

Finally, we considered whether alternative means for cell chain construction could provide similar information. We constructed a cell chain using cells from Reduced-3, denoted ‘Ca-similarity’ for which consecutive cells displayed similar Ca2+ responses. This result makes sense when analysed via the similarity matrices obtained for Ca-similarity did not separate cells into clusters with distinct Ca2+ responses or distinct gene expression profiles (electronic supplementary material, figure S15). However, hierarchical clustering on the parameter posterior distributions from these cells after performing inference on Ca-similarity did not separate cells into clusters with distinct Ca2+ responses or distinct gene expression profiles (electronic supplementary material, figure S15). This result makes sense when analysed via the similarity matrices obtained for Ca-similarity versus a gene expression similarity-based chain (electronic supplementary material, figure S16a,b). For the former, almost all pairs of neighbouring cells did not share similarity in gene expression despite their similarity in Ca2+ response. Whereas for Reduced-3, the gene expression similarity-based chain, all pairs of consecutive cells were similar in gene expression (electronic supplementary material, figure S16a).
4. Discussion

We have presented methods for inferring the parameters of a signalling pathway model, given data describing dynamics in single cells coupled with subsequent gene expression profiling. We hypothesized that via transfer learning we could use posterior information from a cell to inform the prior distribution of its neighbour along a 'cell chain' of transcriptionally similar...
cells. Implemented using Hamiltonian Monte Carlo sampling [42], we discovered that the cell chain construction for prior distributions did indeed lead to faster sampling of parameters. However, these improvements did not rely on the use of gene expression to construct priors: the performance of inference on cells in a chain ordered randomly was equally good. However, cell chains constructed using gene expression similarity contained more information (as defined by their posterior parameter distributions) about Ca²⁺ signalling responses. Clustering the posterior parameters identified important relationships between gene expression and the dynamic Ca²⁺ phenotypes, thus providing mappings from state to dynamic cell fate.

The model studied here is described by ODEs to characterize the Ca²⁺ signalling pathway, adapted from [37,40], consisting of 12 variables and (originally) a 40-dimensional parameter space. This was reduced to 19 parameters in Yao et al. [38] and 16 parameters in our work. Analysis of even a single 16-dimensional posterior distribution requires dimensionality reduction techniques, let alone the analysis of the posterior distributions obtained for thousands of cells. Parameter sensitivity analysis highlighted the effects of specific parameter perturbations on the Ca²⁺ dynamic responses. Indeed, we advocate for the use of sensitivity analysis more generally as means to distinguish and pinpoint the effects of different parameter combinations for models of complex biochemical signalling pathways.

By unsupervised clustering of the posterior distributions, we found that distinct patterns of Ca²⁺ in response to ATP could be mapped to specific variation in the single-cell gene expression. In previous work using similar approaches for clustering [38], posterior parameter clusters predominantly revealed response patterns consisting of responders and non-responders; here we excluded those cells that did not exhibit a robust response to ATP. We were able to characterize subtler the Ca²⁺ response dynamics (described by ‘early’, ‘low’, and ‘late-high’ responders) and predict which transcriptional states give rise to each. This approach is limited since relatively little gene expression variance is explained by an individual model parameter: it may be possible to address this in future work by surveying a larger range of cell behaviours, e.g. by including a wider range of cellular responses or by considering higher-level covariance in the posterior parameter space. It also remains to be tested whether the given model of Ca²⁺ dynamics is appropriate to describe the signalling responses in cell types other than MCF10A cells.

Our ability to fit to the single cells tested came potentially at the expense of an unwieldy model size. With four variables and a 16-dimensional parameter space, the dimension of the model far exceeds that of the data: time series of Ca²⁺ responses in single cells. Without data with which to constrain the three additional model species, we needed to constrain the model in another way. We used an approach of ‘scaling and clipping’ for construction of the priors, i.e. setting ad hoc limits to control posterior variance. More effective (and less ad hoc) techniques could improve inference overall and may become necessary in the case of larger models. These include (in order of sophistication): tailoring the scaling/clipping choices to be parameter-specific; tailoring the choice of prior variance based on additional sources of data; or performing model reduction/identifiability analysis to further constrain the prior space before inference. Constructing priors from cells with similar gene expression also helped to curb the curse of dimensionality: sampling cells sequentially places a constraint on the model. Nonetheless, in the future more directed approaches to tackle model identifiability ought to be considered.

Connecting dynamic cell phenotypes to transcriptional states remains a grand challenge in systems biology. The limitations of deriving knowledge from gene expression data alone [24] have led to the proposal of new methods seeking to bridge the gap between states and fates [52]. Here, making use of technology that jointly measures Ca²⁺ dynamics and gene expression in single cells, we have shown that parameter inference informed by transcriptional similarity represents another way by which we can connect gene expression states to dynamic cellular phenotypes. The statistical framework employed improved our ability to perform parameter inference for single-cell models. We expect that improvements to statistical inference frameworks could be gained by similar approaches applied to other models of nonlinear cellular response dynamics. Current and future technologies combining higher-resolution dynamic cell measurements with high-throughput genomics will provide new data to inform these parameter inference methods and, we expect, lead to new discoveries of means of transcriptional control of biological dynamics.

Data accessibility. All code developed to simulate models and run parameter inference is released under an MIT license at: https://github.com/maclean-lab/singlecell-parinf. Parameter inference was developed in Python 3.6 and Stan 2.19. Posterior analyses were developed in Python 3.8. Ca²⁺ data and MERFISH data (processed files) are also available from the GitHub repository: https://github.com/maclean-lab/singlecell-parinf.

The data are provided in electronic supplementary material [33].

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References

1. Wilkinson DJ. 2020 Stochastic modelling for systems biology, Chapman & Hall/CRC mathematical and computational biology, 3rd edn, first issued in paperback edition. Boca Raton, FL: CRC Press.
2. Hoops S et al. 2006 COPASI—a complex pathway simulator. Bioinformatics 22, 3067–3074. (doi:10.1093/bioinformatics/btf485)
3. Liepe J, Filippi S, Komorowski M, Stumpf MPJ. 2013 Maximizing the information content of experiments in systems biology, PLoS Comput. Biol. 9, e1002888. (doi:10.1371/journal.pcbi.1002888)
4. Wame DJ, Baker RE, Simpson MJ. 2019 Simulation and inference algorithms for stochastic biochemical reaction networks: from basic concepts to state-of-
15. Periasamy M, Kalyanasundaram A. 2007 SERCA pump isoforms: their role in calcium transport and calcium signaling in prokaryotes. Cell Calcium 57, 151–165. (doi:10.1016/j.ceca.2012.04.006)

16. Ma LH, Webb SE, Chan CM, Zhang J, Miller AL. 2009 Approximate Bayesian computation scheme for parameter inference and model selection in dynamical systems. J. R. Soc. Interface 6, 187–202. (doi:10.1098/rsif.2008.0172)

17. Balaji R, Bielmeier C, Harz H, Bates J, Stadler C, Schöler U. N2a cells express two classes of ERK1/2 phosphorylation in a Pax6+/−/− mouse model of epithelial wound-healing delay. BMC Biol. 4, 27. (doi:10.1186/1471-2121-4-27)

18. Mallet J, Heygi P. 2014 Calcium signaling in pancreatic ductal epithelial cells: an old friend and a nasty enemy. Cell Calcium 55, 337–345. (doi:10.1016/j.ceca.2014.02.004)

19. MacLean AL, Hong T, Nie Q. 2018 Exploring intermediate cell states through the lens of single cells. Curr. Opin. Syst. Biol. 9, 32–41. (doi:10.1016/j.cosbi.2018.02.009)

20. Moffitt JR, Zhuang X. 2016 RNA imaging with multiplexed error-resistant fluorescence in situ hybridization (MERFISH). In Methods in enzymology (eds Filonov GS, Jaffrey SR), vol. 572, pp. 1–49. Amsterdam, The Netherlands: Elsevier.

21. Eng C-HL et al. 2019 Transcriprome-scale super-resolved imaging in tissues by RNA seqFISH+. Nature 568, 235–239. (doi:10.1038/s41586-019-1049-y)

22. Stumpf PS et al. 2020 Mammalian genes are transcribed with widely different bursting kinetics. Science 332, 472–474. (doi:10.1126/science.1198817)

23. Balaji R, Bielmeier C, Harz H, Bates J, Stadler C, Schöler U. N2a cells express two classes of ERK1/2 phosphorylation in a Pax6+/−/−/− mouse model of epithelial wound-healing delay. BMC Biol. 4, 27. (doi:10.1186/1471-2121-4-27)

24. Mallet J, Heygi P. 2014 Calcium signaling in pancreatic ductal epithelial cells: an old friend and a nasty enemy. Cell Calcium 55, 337–345. (doi:10.1016/j.ceca.2014.02.004)

25. Dupont G, Combettes L, Bird GS, Putney JW. 2011 Calcium oscillations. Cold Spring Harbor Perspect. Biol. 3, a004226–a004226. (doi:10.1101/cshperspect.a004226)

26. Periasamy M, Kalyanasundaram A. 2007 SERCA pump isoforms: their role in calcium transport and disease. Muscle Nerve 35, 430–442. (doi:10.1002/mus.20745)

27. Ma LH, Webb SE, Chan CM, Zhang J, Miller AL. 2009 Establishment of a transitory dorsal-biased window of localized Ca2+ signaling in the mid-blastula transition in zebrafish embryos. Dev. Biol. 327, 143–157. (doi:10.1016/j.ydbio.2008.12.015)

28. Balaji R, Bielmeier C, Harz H, Bates J, Stadler C, Hildebrand A, Classen A-K. 2017 Calcium spikes, waves and oscillations in a large, patterned epithelial tissue. Sci. Rep. 7, 42786. (doi:10.1038/srep42786)

29. Dominguez DC, Guragain M, Patrauchan M. 2015 Calcium binding proteins and calcium signaling in prokaryotes. Cell Calcium 57, 151–165. (doi:10.1016/j.ceca.2014.02.006)
49. Gutenkunst RN, Waterfall JJ, Casey FP, Brown KS, Myers CR, Sethna JP. 2007 Universality sloppy parameter sensitivities in systems biology models. PLoS Comput. Biol. 3, e189. (doi:10.1371/journal.pcbi.0030189)

50. Varma A, Morbidelli M, Wu H. 1999 Parametric sensitivity in chemical systems, 1st edn. Cambridge, UK: Cambridge University Press.

51. Komorowski M, Costa MJ, Rand DA, Stumpf MPH. 2011 Sensitivity, robustness, and identifiability in stochastic chemical kinetics models. Proc. Natl Acad. Sci. USA 108, 8645–8650. (doi:10.1073/pnas.1015814108)

52. Qiu X et al. 2022 Mapping transcriptomic vector fields of single cells. Cell 185, 690–711. (doi:10.1016/j.cell.2021.12.045)

53. Wu X, Wollman R, MacLean AL. 2023 Single-cell Ca²⁺ parameter inference reveals how transcriptional states inform dynamic cell responses. Figshare. (doi:10.6084/m9.figshare.c.6662626)