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Information Theory as an experimental tool for integrating disparate biophysical signaling modules

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Abstract

There is a growing appreciation in the fields of Cell and Developmental Biology that cells collectively process information in time and space. While many powerful molecular tools exist to observe biophysical dynamics biologists must find ways to quantitatively understand these phenomena at the systems level. Here, we present a guide for application of well-established information theory metrics to biological datasets and explain these metrics using examples from cell, developmental and regenerative biology. We introduce a novel computational tool (CAIM) for simple, rigorous application of these metrics to timeseries datasets. Finally, we use CAIM to study calcium and cytoskeletal Actin information flow patterns between *Xenopus laevis* embryonic animal cap stem cells. The tools that we present here will enable biologists to apply information theory to develop systems level understanding of a diverse array of experimental systems.

Introduction: challenges of studying biology at the systems level

It is an inherent limitation of science that the experimentalist is constrained by their tools. The phenomena that we study do not share these limitations, however. When we conflate the constraints of our methodology with the constrains of our system we lose sight of the importance of that which we cannot yet measure. During the 20th century the establishment of the Central Dogma and the advances in genetics and molecular biology that it enabled allowed us to perturb and quantify biological systems at a fundamental level. During the 21st century it has become increasingly clear that biology is not so easily reduced to its component parts. As we expand our understanding of biology from cells and pathways to tissues and systems we will have to move beyond our clean linear ideas of causality like 'signaling pathways' and 'genotype determining phenotype' to do justice to the complexity of biological information processing [1-4].

Three main barriers exist to the rational discovery of interventions based on simple systems-level causal models in cell and developmental biology. First is the problem of time. Many manipulations, including drug treatments and genetic knockouts, constitutively activate or inhibit specific pathways but in so doing remove any information encoded in the dynamics of the signal. There is a growing body of evidence that such dynamics are crucial to proper cell signaling with specific outcomes encoded by different signal dynamics.[5-9]. Importantly, temporal interactions are not merely inconvenient artifacts, but core to the very idea of causality @. Emerging tools like optogenetics [7,10] allow us to manipulate systems with high temporal resolution, but new analytical approaches will be necessary to implement these tools at the systems level in non-neural systems.

Second is the problem of integration. Due to technical limitations, it is standard to manipulate a single pathway at a time and test for effects on a targeted set of outcomes. But biological systems are built to be robust to just this type of insult manifesting as spontaneous mutations and thus we are often limited in our ability to understand and control systems governed by redundancy and integration. Further, the specificity of independent 'pathways' is confounded by the tendency of multiple different pathways to converge into a small set of 'secondary messengers' (Figure 1)[11-14]. Understanding

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how diverse inputs are integrated will dramatically improve our ability to predict and control biological systems, but will require supplementing beyond classical reductionist methodology with new integrative approaches.

Third is the problem of computation. It is clear that studying an individual neuron is entirely insufficient to understand functioning of the nervous system [15]. Neuroscientists overcame this problem by applying tools like Positron Emission Technology (PET), fMRI and calcium imaging to measure system level behavior of neural tissues. In non-neural systems too, it is becoming increasingly clear that many aspects of tissue level function are irreducibly complex and require new tools that go beyond simple linear pathways commonly used in molecular genetics models [16-19]. The above techniques are increasingly being adopted by non-neural biologists, but without analytical tools to deal with the data that they produce that cannot be used to their fullest potential.

Here we propose applying Information Theory as a useful approach to these three problems. Information Theory provides methodology for quantifying correlative and causative interactions between different variables. Though it forms the basis of computation, information theory is not to be confused with mathematical modeling. Indeed, Information Theory is unique in being model-agnostic with respect to the implementation details of the system being analyzed. It can readily be applied to extremely different systems, including gene-gene interactions, genotype-phenotype interactions to cell-cell interactions and beyond, requiring very few prior assumptions of how those systems work (i.e., being compatible with a wide range of mechanisms). By allowing researchers to map information flow between cells and pathways, Information Theory shows promise to facilitate systems level understanding of biological phenomena for which molecular reductionism has failed, and to leverage the emergent power of tissues for synthetic biology applications[17]. Indeed, the transformative nature of Information Theory should not be underestimated: Shannon's 1948 paper [20] directly ushered in the Information Age and the global integration that defined it, and his formalism is the basis for the analysis that we present here.

Here, we describe useful information theory metrics and provide analogies and examples from known biological systems. Our goal is to describe a variety of Information Theory techniques and to illustrate how they may be applied by experimental biologists (Figure 2). We explore concepts with analogy and re-evaluation of experimental systems, but not delve too deeply into the underlying mathematics of these concepts, since each is a well-accepted technique supported by rigorous mathematical principles that are described in detail in the cited references.

We then introduce a novel tool, CAIM, designed to allow biologists to easily and rigorously apply several of the metrics we discuss to their own data. We demonstrate how CAIM can be applied to describe information flow dynamics at the systems level of calcium, a key signaling node, and Actin, a key component of the cytoskeleton that directly effects many biological processes including morphogenesis, the early embryonic *Xenopus laevis* stem cells. Finally, we integrate these data to propose a model of the system-level functions of these signaling modalities in this primitive tissue.

Information Metrics

The real power of Information Theory comes from its rigorous mathematical foundation. Science is fraught with beautiful ideas like 'memory,' 'consciousness' and

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even 'life' that seem intuitively clear but are too nebulous to withstand rigorous scientific scrutiny. Information is not one of these ideas. In 1948 Claude Shannon famously defined Information using the formula $I=-\Sigma p\log(2)p$, which we describe in greater detail in Figure 3A. This formula may seem familiar, and indeed it is very similar to the formulation of thermodynamic entropy. Indeed, the two concepts are closely related. Consider two libraries, one well-organized (low thermodynamic entropy) and one very cluttered (high thermodynamic entropy). Describing the location of every book in a well-organized library requires very little information ("They are alphabetical by title starting here"), while describing the location of every book in a disorganized library requires a lot of information ("Melville is there, Shakespeare over here, Seuss down in the basement" etc.). And, just as Thermodynamics allows us to quantify and apply a seemingly nebulous concept like 'order', Information theory allows us to quantify communication in systems.

Quantifying information can aid in one of the greatest challenges for the experimentalist: deciding which tool to use to study a system. Imagine that we have 3 fluorescent reporters for the same metabolite with different sensitivities (Figure 3B,C). The first is active 10% of the time (I=0.47 bits), the second is active 90% of the time (I=0.47 bits), the third is active 50% of the time (I=1.00 bit). Even though we'll get the most signal from the second reporter we'll get the most information from the third. Most of the metrics that we describe below are bounded by the amount of information of a variable, and thus it is wise to tailor our approaches to extract the maximum amount of information from our system.

Mutual information

Mutual information (MI) is a measure of how much our uncertainty of one variable is decreased by knowing the state of another variable [20] (Figure 4A). Imagine that Alice and Bob always arrive to work at 8:00 AM. The arrival times of these people will have very high mutual information: we can usually tell if Bob is around by knowing whether or not Alice is around. We cannot, however, predict anything about the relationship between Alice and Bob. It may be that they live together and carpool; it may be that they live separately but arrive on the same bus; or it may be that both of their shifts start at 8:00 AM. MI will not give us any insight into the reason that the two arrive at the same time.

From a practical standpoint, establishing mutual information between two factors can be very important even without knowing the mechanistic interaction between the two, especially if one of the factors is difficult to measure. This is the core of what physicians do in diagnostic assays: measure phenomena with high mutual information to disease states. It isn't necessary to understand why heart attacks cause intense pain in the left arm, it doesn't really matter if we can reliably use the high MI between the two to quickly diagnose heart attacks. A biological example of this utility from our own work can be seen in symmetrical effects of amputation [21]. We observed that upon amputating a developing frog limb, the contralateral (un-damaged) limb would stain strongly with the potentiometric dye DiBac at approximately the proximal-distal location as the site of amputation, consistent with these two distant sites had surprisingly high mutual information post-amputation (Figure 4B). The cells of one limb had information about the state of cells in the opposite limb; this finding revealed an unexpected phenomenon even before investigations of the mechanism of the correlation, for example suggesting the possibility of surrogate-site diagnostics.

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As a warning to the experimentalist, while MI is useful as an initial guide to forming hypotheses, as it can also reveal relationships that are "artifacts" in the sense that they do not represent interesting causal connections. For example, if the light source used in a microscopy experiment fluctuates over time then two nearby cells will demonstrate high MI, but this MI will not reflect biologically meaningful interactions. Despite these caveats MI is a powerful 'first step' for information theory analysis because its model agnostic nature facilitates discovery of unexpected phenomena.

Delayed Mutual Information

An obvious limitation to the applicability of Mutual Information is its inability to quantify events that are highly correlated but separated in time. This prevents MI from detecting causal interactions in which, by definition, one event must precede another. A simple solution to this problem is to temporally offset the expected 'sending' variable from the 'receiving' one. If it takes a signal four second to propagate from Cell 1 to Cell 2, then measuring the state of Cell 1 should accurately predict the state of Cell 2 four seconds later(Figure 5A). Returning to the example of the beating heart, imagine two people with similar resting heartrates. Assuming their heart rates are not in phase there will be a high degree of Delayed MI between the two: We can accurately predict when Person A's heart is going to beat if we known when Person B's heart beats.

An example of the potential pitfalls of delayed mutual information can be found in the elegant experiments of Isabel Palmeirim and colleagues in their discovery of the segmentation clock. They noticed waves of *hairy* gene expression that seemed to emerge from the tip of the tail and move anteriorly until they eventually formed a new body segment[22]. In Information Theory terms, they essentially detected high delayed mutual information between cells along the anterior-posterior axis, which suggested that the posterior cell was directly causing the state of its anterior neighbor. They cleverly tested this hypothesis by ablating the 'sender' cells in the tailbud (Figure 5B). Surprisingly, the *hairy* wave still manifested, demonstrating that the causal prediction from their delayed mutual information analysis was incorrect and inferring that the two cells were oscillating independently.. Interestingly, future work [23-25] showed that these cells do require some input from their neighbors to oscillate correctly. from this example it is clear that more refined tools are necessary to satisfyingly measure causality, especially in biological systems that cannot be intervened upon as readily as the segmenting tailbud.

Active Information Storage (AIS)

The segmentation clock example above exemplifies the importance of knowing the mutual information between a cell's current and past states. If a cell's state is heavily informed by its past this suggests that it is independent and less governed by collective dynamics. We can measure this by measuring the DMI between a cell's dynamics and a time-delayed version of these same dynamics to generate our next metric: Active Information Storage.

Consider a beating heart (Figure 6A): We can very easily predict when contractions are going to happen by observing the previous beating rate. Indeed, periodic signals manifest throughout biology, from the vertebrate segmentation clock to circadian rhythms[26]. Active Information Storage (AIS) is a formulated form of MI between a timeseries and a past version of itself [27-30].

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Moreover, quantifying changes of AIS can provide important insights into the behavior of a biological system. If the AIS of beating heart drops slightly, this may indicate that its host organism is exercising or experiencing stress that gradually changes the beat frequency(Figure 4B). Likewise, if AIS drops in a non-cardiac cell this loss of autonomous predictive power suggests that the cell is being affected by an external force @. We cannot infer what is causing this change, but by identifying and quantifying this interaction we can design experiments to determine its molecular mechanism. If the AIS of a heart drops dramatically this may suggest a serious pathology like arrythmia or even fibrillation. In turn, dramatic loss of AIS in a non-cardiac cell may suggest an important transition. During differentiation a cell may become fundamentally different from its previous state thus reducing the predictive power of its past for its future. AIS could also drop dramatically if a cell is integrated into a super-cellular collective, as the collective may have substantial input on the state of the cell and only observing the past states of the cell may lose predictive value.

High AIS values, therefore, may suggest that cells are behaving more independently and less collectively. If this is true then experimentally isolating the cells should have a minimal effect on the dynamics. Low AIS values, in contrast, suggest that the dynamics are being largely governed by other forces that can be investigated by our next metric: Transfer Entropy.

Transfer Entropy

Inferring causality is one of the main goals of experimentation, but can also be one of the most challenging. Our standard approach is to perturb the system and see how it changes and to then attribute the changes to the perturbation. But these perturbations are often of non-physiological magnitude, and despite our best efforts at control may be confounded with technical artifacts. Alternatively, we may observe a system over time without perturbation and observe the timing of events, effectively estimating the delayed mutual information between them, but this approach is prone to conflation of correlation with causation. In information theory the best current tool for inferring statistical causation is called Transfer Entropy (TE)[31,32]. Recall that the terms Entropy and Information are often used interchangeably, and thus TE can be thought of as Transfer of Information. TE is the amount of information about one variable gained from knowing the state of a second variable beyond what is learned from the history of the first variable(Figure 5A).

Let's say that Alice always arrives to work at 7:50 and Bob always arrives at 8:00. It may well be that Alice and Bob drive in together, Alice lets Bob off at the entrance (on account of his bad knee) and goes to park. It may be that Bob hates Alice, and always makes sure to arrive later than her to avoid running into her. In both of these cases it would be fair to say that Alice's arrival time causes Bob's arrival time. However, it could also be that Bob just shows up at 8:00 because that's when his shift starts. Because we can predict when Bob is going to arrive without knowing anything about Alice we can't really conclude that her arrival time is causing his arrival time, despite reliably preceding it and allowing us to accurately predict.

, While transfer entropy is a powerful tool, but its broad definition of 'causality' may ring hollow to an experimentalist. We spend our lives intervening upon systems and measuring outcomes to infer causal relationships. TE excels at analyzing systems that

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cannot be readily manipulated, and can help us decide which systems warrant development of experimental interventions or translation into tractable model systems. One we can manipulate our system, we can move to our next metric: Effective Information.

Effective information (EI)

Effective Information (EI) is a formal way of measuring causal interactions between an experimental manipulation and a system [33]. To measure EI one sets the state of each variable and measures the consequences on the future states of the system (Figure 7A). This technique is conceptually very similar to many experimental biological approaches. In a reverse genetic screen, one individually mutates a set of genes (thus setting their state to 0 for null mutations, <1 for hypomorphic mutations and >1 for hypermorphic mutations) and observe the phenotypic consequences on the phenotype of the organism (Figure 7C). Importantly, there is rarely a linear 1 to 1 correlation between a single gene and the phenotypic outcome of a complex biological system. Biologists refer to this phenomenon as 'incomplete penetrance'.

The discovery of 'Instructor Cells' in the skin of *Xenopus* embryos provides a nice example of a system with Effective Information (Figure 7B. Depolarizing a specific subset of epidermal cells causes the entire embryo to become hyperpigmented [34]. Interestingly, this hyperpigmentation exhibits a binary 'all-or-nothing' pattern within each animal, indicating that depolarizing the instructor cells affects the system as a whole [35,36]. In information theory terms, they measured the EI between the 'instructor cells' and pigmentation of the embryo system by injecting noise into the instructor cells by either depolarizing them or leaving them as unmanipulated controls then estimating the mutual information between the state of the instructor cell and the state of the pigmentation. If they had chosen to depolarize a set of 'non-instructor cells' instead they would have found very little mutual information between the state of the manipulated cells and pigmentation since the state of the non-instructor cells does not predict the pigmentation state.

Importantly, EI as with all the metrics presented here is a continuous, not binary, metric, and thus gives us a framework for quantifying the degree of causal relationship between a manipulation and a phenotypic output. This quantification has the potential to address a substantial problem that has arisen from molecular reductionism. Different researchers apply different methods to similar questions and report any statistically significant findings regardless of effect size. These findings are then compiled in pathway diagrams in which each experiment is recorded as an arrow, and all arrows are weighted equally regardless of whether the manipulation caused a 10% change or a 90% change and regardless of the technical differences between the techniques used. Because of its model free nature, EI has the potential to unify these diverse experimental findings to bits, which can then be compared and compiled.

Consider two researches studying the same protein. One finds that an intervention increases phosphorylation of Protein X 80% of the time via Western Blot. The other finds that the same intervention induces ectopic tails 5% of the time in mice. It is tempting to combine these data to say that 'This intervention increases Protein X phosphorylation and causes Phenotype Y,' but doing so strips the data of important causal information. EI can be applied just as readily to phosphate groups and tail numbers and thus allows us to integrate these orthologous techniques.

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Information theory analysis of calcium and Actin dynamics in *Xenopus laevis* stem cells

Secondary messengers and primary nodes

Neuroscientists during the 1990's faced the same fundamental problem that non-neural scientists are facing today: How can we measure information dynamics between cells when the mechanisms that transmit this information are fast and subtle? Rafael Yuste and colleagues solved this problem by using calcium signaling to great effect as a proxy for action potentials [37-39]. Calcium also functions as an important secondary messenger in many diverse non-neural systems [11,40-43]The approach of applying IT metrics to non-neural calcium signaling is further supported by the observation of striking long-range coordinated calcium patterns that move across many different cells [44]. Information Theory measures states of variables, and as discussed in the preceding section, it is convenient for our purposes to coarse-grain the complexity of a cell to a single binary on or off calcium state signal (Figure 3C). Once interactions have been identified and measured we can employ our prodigious arsenal of molecular tools to determine the molecular mechanisms and functional consequences of these patterns.

It is unreasonable to assume that calcium levels alone is sufficient to define the state of a cell. Fortunately, the factors that make calcium an appealing cell state reporter can also be found in other secondary messengers. The RAS/ERK signaling pathway, as one example, also integrates input from many different signaling pathways and displays large scale emergent dynamical patterns [45,46]. Indeed, it may be time to consider promoting these 'secondary messengers' to 'primary nodes' that do not merely transduce signals but integrate information from many different input pathways to determine the behavior of a cell.

There is a growing body of evidence that pulsatile dynamics in the Actin cytoskeleton play important roles during morphogenesis[47]. These cytoskeletal dynamics have been described in a variety of tissues, including the *Xenopus laevis* primitive animal cap embryonic stem cell tissue[48]. Further, pulsatile Actin dynamics have been linked with calcium dynamics in several developmental systems including mast cells[49] and the developing *Xenopus* neural plate[50]. In order to demonstrate the profound flexibility of Information Theory we apply these metrics to both calcium signaling and Actin pulsation. These signaling modalities are very different at the mechanistic level. Calcium is an ion that mediates communication through several signaling pathways, while Actin is a structural cytoskeletal protein that contributes to a cell's shape and facilitates its mechanical interaction with its environment[40,47]. We have chosen to analyze these very different signaling modalities to demonstrate how the model-free nature of Information Theory allows us to study interactions between diverse biological phenomena. We do so in explant *Xenopus laevis* animal cap embryonic stem cells. These cells demonstrate both endogenous Actin and calcium dynamics, but it remains unclear how they interact at the systems level and what function, if any, they play during early development. By applying information theory to the dynamics of these signaling modalities between and within minimally manipulated tissue explants we aim to explore the degree to which these cells behave collectively and what roles these modalities play in this collectivization.

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CAIM (Calcium Imaging)

We introduce and apply here a novel software package called CAIM (Calcium Imaging) designed to calculate AIS, MI and TE using the Inform library [51,52]. CAIM was designed as a collaborative effort between biologists and mathematicians to allow researchers to apply these metrics to easily and rigorously apply Information Theory to real biological data sets. CAIM takes timeseries datasets (Figure 8A) and plots Intensity vs. time for ROIs that can be drawn within the CAIM program (Figure 8B). We generated mosaic timeseries in FIJI with 9 ROIs in four quadrants: Real Actin data in the top left, real calcium data in the bottom left, randomized Actin data in the top right and randomized calcium data in the bottom right. This setup allows for an easy analysis between different conditions.

CAIM enables binarization of timeseries data sets via a variety of thresholding techniques and displays the binarized timeseries (Figure 8C). This function enables researchers to easily assess how accurately the applied binarization represents their data. For our analysis we have elected to binarize our timeseries using the mean threshold as a cutoff between signal and noise.

Once the signal is binarized CAIM can be used to calculate AIS, MI and TE between ROIs. Results are displayed in tables with individual ROIs being identified by the color used to demarcate them on the timeseries window. Because AIS does not involve comparison between conditions a single value is calculated per ROI. Pairwise comparisons are made between each ROI for MI and TE. Because MI is not directional only a single value per pair is displayed. Measurement of an ROIs MI with itself provides the information content of the timeseries as defined by the binarization conditions. As TE Is directional CAIM produces a separate value for each direction of each pairwise comparison. Source ROIs determine the row of the table and target ROI determines the column.

Each data point is statistically compared against a randomized group of 1000 random time series and values with $p<0.05$ are colored blue. This provides an easy way to visually detect trends.

Technical challenges

Due in part to its model-free nature Information Theory based analysis does not readily distinguish between biologically interesting signal fluctuations and technical artifacts. Strong photobleaching, for example, will cause all regions to decrease signal in parallel, and such timeseries will have high Mutual Information that does not reflect biological behavior. Much of the GCaMP8S data that we present here relies on comparatively weak sub-threshold fluctuations that may be substantially affected by imaging noise which may be registered as signal due to our Mean Threshold binarization approach. Further, these images were collected using single-plane confocal microscopy, which provides relatively fast clear images, but only visualizes a single surface of the cell and thus may miss important factors outside the imaging plane. These potential confounds are limitations to be kept in mind and addressed in future work.

Materials and Methods

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Animal Husbandry:
Fertilized embryos were raised at 14°C in 0.1X Marc's Modified Ringer solution until the onset of gastrulation. Frogs were maintained in accordance with IACUC protocol M2020-35.

Microinjections: Embryos were injected into 2-4 cell embryos, with jGCaMP8S[53] being injected at 600 ng/µl and LifeAct-mCherry being injected at 100 ng/µl. mCherry-Lifeact-7 was a gift from Michael Davidson (Addgene plasmid # 54491 ; http://n2t.net/addgene:54491 ; RRID:Addgene_54491)

Explant culture: 8-chambered Nunc Lab-Tek II Chambered coverglasses that had been coated with 0.1 mg/mL human plasma Fibronectin (Sigma Aldrich, F2006) for 60 minutes at 37°C in 1XPBS[54]. The dishes were then washed 1X with PBS, 1X with Danilchick's For Amy (DFA) buffer that was replaced with fresh DFA buffer[54]. The superficial ectoderm of the animal pole of Nieukoop and Faber Stage 10 embryos was surgically removed in 0.1X Marc's Modified Ringer solution[55]. The deep ectoderm was then isolated and transferred to the Fibronectin coated coverglass chambers. Explants were allowed to adhere and spread overnight at 18°C and imaged the following day.

Imaging: Explants were imaged on a Leica Stellaris Sp8 confocal microscope for 1 hour each with a capture rate of one frame per minute. GCamP8S and LifeAct-mCherry were imaged in parallel with separate excitation wavelengths and separate detectors. Specific imaging conditions are available in the representative metadata file (Supplementary Methods 1).

Image Processing: 9 100 pixel by 100 pixel ROIs were selected per explant timelapse. ROIs were chosen that A.) would remain in a single cell despite cell motion during the timelapse and B.) were in a domain of the cell that appeared to display Actin dynamics. Each explant contained a mixture of ROIs selected at the explant periphery and more internal. Effort was made to choose ROIs from juxtaposed cells where possible with cells exhibiting prohibitive cell motion being discarded.

Image randomization: 18 arrays of numbers 1-60 were generated in Microsoft Excel. A different array was used for each ROI, and separate arrays were used for the two channels. The 18 arrays and randomization macros are attached in Supplementary Methods 2.

Statistics: Statistics were performed using GraphPad Prism. Pairwise significance was assayed using Mann-Whitney tests because we could not assume Gaussian distributions of our data sets. To compare real data sets to multiple randomized data sets pairwise comparisons were used instead of ANNOVA tests to prevent false positives resulting from differences between the randomized data sets, and the null hypothesis was only rejected if the real data set was significantly different from each randomized data set.

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Information Theory Analysis: Active Information, Mutual Information and Transfer Entropy were measured using the novel CAIM software package[51]. CAIM facilitates application of the Inform software package [52] to timeseries data sets.

Results

Active Information Storage (AIS): Both Actin and Calcium dynamics are affected by their previous states

We measured the Active Information Storage (AIS) of Actin and calcium dynamics in each of our ROIs compared with randomized control timeseries (Figure 9A-D, Supplemental Movie 2-4). We imaged 9 ROIs on each of 10 explants which were collected from three separate rounds of injection. For both signal types the AIS is significantly higher than the randomized control, suggesting that both Actin and calcium dynamics are informed by their previous behavior. This conclusion is consistent with our qualitative observations. Actin pulses seem to be somewhat consistent in duration with a refractory period between pulses. The calcium dynamics, in contrast, seem to change gradually over time, but in both cases the future behavior of the signal is well predicted by its past.

Interestingly, Actin dynamics have significantly higher AIS than calcium signaling. This finding suggests that Actin dynamics are better correlated with their own history than are calcium dynamics.

Both Actin and calcium dynamics correlate between nearby cells

To quantify correlative relationships within Actin and calcium dynamics in nearby cells we measured pairwise MI between all combinations of nearby cells in each of our explants for both channels imaged (Figure 8A,9E). We then compared these values with random datasets generated by measuring MI between these ROIs and randomized ROIs generated from the same explant. We further measured MI between the randomized ROIs. Both the calcium and Actin dynamics showed significantly increased MI compared to the randomized data sets generated from their respective channels. Moreover, calcium dynamics showed significantly increased MI compared to Actin dynamics. These data demonstrate that information is shared in both the Actin and calcium dynamics of nearby cells, and suggest that more information is shared between cells via the channel of calcium signaling than via Actin dynamic signaling.

Calcium dynamics, but not Actin dynamics, demonstrate information transfer between nearby cells

To quantify causal relationships within Actin and calcium dynamics we measured pairwise TE between all combinations of nearby cells in each of our explants for both channels imaged (Figure 9F). Because TE, unlike MI, is directional we measured TE in both directions for each cell pair. We also generated three random data sets: one in which the sending cell timeseries was randomized, one in which the receiving cell timeseries was randomized, and one in which both were randomized.

We did not detect any significant difference in TE between the real Actin dynamics dataset and either the ‘randomized sender’ or the ‘randomized receiver’ group, and the real data contained significantly less TE ($p=0.0012$, Mann-Whitney test) than the ‘random

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sender and random receiver' group. We thus conclude that there is no detectable transfer of information from one cell to another in the Actin signal.

In contrast, we do detect significantly higher TE in the real calcium dynamics dataset than in either of the three randomized controls. This finding suggests that information may be passed from one cell to another via the calcium channel but not the Actin channel.

Actin and calcium dynamics share information

Having measured MI and TE between cells for both channels, we next sought to measure the information dynamics between channels. We began using the same approach that we used for the individual channel analysis: we measured pairwise MI between the two channels for each pair of ROIs (10A). We found significantly higher MI between real Actin and calcium dynamics than randomized versions of these datasets, though the MI between channels was significantly lower than the MI within the same channel between ROIs. Together these data suggest that Actin and calcium dynamics are not independent in animal cap explants

Actin dynamics have significant TE to calcium dynamics, but calcium dynamics do not have significant TE to calcium dynamics.

We followed our inter-channel MI analysis by measuring TE between pairs of ROIs. We found that Actin dynamics had significant TE to calcium dynamics, but that this TE was not bidirectional (Figure 10B). This was somewhat surprising, as we had previously found that Actin does not have significant TE between cells while calcium does. This data suggests a complex, directional relationship between Actin and calcium in animal cap explants.

Intracellular Mutual Information (MI): Actin and calcium dynamics share information

To more highly resolve the information dynamics between Actin and calcium we measured MI within each individual ROI in order to disentangle intracellular and intercellular effects The real data set has significantly greater MI than the randomized data sets, indicating that these two signals share information within a single cell.

Intracellular Transfer Entropy (TE): Actin causally affects calcium dynamics, but calcium dynamics do not causally affect Actin dynamics

Finally, we measured TE between the Actin and calcium channels for each ROI(Figure 10C). We found that, consistent with our previous pairwise ROI data, on an individual cell basis Actin dynamics demonstrate significantly higher TE to calcium dynamics than calcium dynamics due to Actin dynamics. Likewise TE from Actin to calcium dynamics is significantly higher than the randomized controls, while TE from calcium to Actin is insignificantly different from random controls. These data further support our conclusion of a directional information flow from Actin dynamics to calcium dynamics and suggest that this interaction happens at the individual cell level.

Discussion:

Many aspects of information flow are consistent between Actin and calcium dynamics. Both modalities display significant AIS, and both display significant MI with

neighboring cells. These data suggest that both modalities are largely affected by their own histories but, at the same time, share information with their neighbors.

Information flow patterns in Actin and Calcium dynamics share some key differences, however, indicating that they are not simply manifestations of a single signaling regime. Actin dynamics display significantly greater AIS than calcium dynamics do, while calcium dynamics display greater MI between cells than Actin dynamics do. Taken together these data point to a greater role for Actin dynamics in maintenance of a cell's state and calcium playing a greater role in mediating coordination between cells. This trend is further supported by our TE analysis, which shows significant information transfer in the calcium modality but not in the Actin modality. Information analysis of crosstalk between the Actin and calcium reveals that Actin dynamics affect calcium dynamics, but calcium dynamics do not detectably affect Actin dynamics (Figure 10A).

We propose (Figure 10D) that Actin establishes relatively stable (high AIS) cell collectives (high MI) but does not directly mediate information flow between cells (low TE). This interpretation is consistent with the role of supracellular Actin cables in tissue compartmentalization [56] , and suggests that this type of compartmentalization may manifest at some level very early even in seemingly poorly organized tissue . Calcium, in contrast, does mediate information flow between cells (high MI and TE), but is less persistent than Actin architecture (relatively low AIS). Finally, because Actin does affects calcium dynamics (high TE, Figure 10A) we propose that Actin dynamics establish compartments through which calcium mediated information flows.

Ultimately, the goal of this analysis is to inform experimental interventions to allow us to understand and control tissue behavior at the systems level. The Effective Information framework discussed in the introduction gives us a quantitative way to translate our findings from simple observation to causal intervention. Because calcium dynamics but not Actin dynamics display significant intercellular transfer entropy, we predict that perturbations in one cell are likely to affect the calcium dynamics of distant cells, but not the Actin dynamics. We predict that intervening upon Actin dynamics will, however affect calcium dynamics, even in nearby cells, but that affecting Actin dynamics should not perturb calcium signaling. Thus, the analysis that we provide here is a key step in establishing a robust, quantitative and experimentally supported systems-level understanding of collectivity in embryonic stem cells.

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Figure Legends

Figure 1: (A) Cell signaling is often conceptualized as pathways between signals and target genes with secondary messengers as intermediates. (B) Because the same secondary messengers are used in many different pathways, it may be more appropriate to think of them as primary nodes integrating diverse signaling regimes.

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Figure 2: Information is a formally defined value (A) that increases as variables spend more time in multiple states (B). Signals that frequently shift between 'active' and 'inactive' states (green) contain more Information than signals that are either always on (blue) or always off (red).

Figure 3: Suggested workflow for deciding which Information Theory metrics to apply based on qualitative observations of a system.

Figure 4: (A) Mutual Information measures the information that knowing the state of one variable provides about a second variable. (B) Mutual Information can be used to detect novel communication channels, as in the case of contralateral bioelectric injury signals. Redrawn after [21].

Figure 5: (A) Active Information Storage measures how well a variable's past predicts its current state. (B) A healthy heart should have relatively high Active Information Storage, while an unhealthy heart in which periodicity is pathologically perturbed show have lower AIS.

Figure 6: (A) Delayed mutual information measures the predictive power of one variable to another variable's future allowing for detection of causal interactions. Transfer entropy is a more powerful approach that considers predictive power of the receiving variable's own history, and thus avoids inferring causation from time delayed correlative interactions. (B) The vertebrate segmentation clock is a biological example of the incorrect interpretations that can arise from failing to account for a cell's history. Waves of gene expression appear to migrate posterior to anterior in the growing tail, but will form even when the apparent 'sending' cells are ablated, indicating that they result from intrinsic oscillations within the cells.

Figure 7: (A) Effective information measures the predictive power of interventions over a system. (B) When 'Instructor cells' are set to a particular state, the state of the system can be well predicted and thus these cells have high Effective Information with the system. Setting the state of non-instructor cells, however, does not well predict the state of the system, indicating low effective information between the cell and the system. (C) An approach similar to Effective Information is used in reverse genetic screens in which each candidate gene (variable) is set to a specific state via mutation and the predictive power of this mutation over a trait is calculated.

Figure 8: (A) CAIM provides users a GUI to select regions of interest from which it will extract timeseries data (B) that can then be binarized (C). AIS, ME and TE can then be calculated between each ROI and displayed in tables with statistically significant values indicated in blue.

Figure 9: **Information metrics within each signal**. Representative frame of LifeAct-mcherry (A) GCAMP8S (B) and an overlay (C) of of the two channels from one of the explants imaged. AIS is significantly higher in real vs randomized datasets for both the

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Actin and calcium signals, and AIS for the Actin signal is significantly higher than for the calcium signal (D). Mutual information between ROIs within the same timeseries is higher than for the randomized datasets, and MI is higher between ROIs for the calcium signal than for the Actin signal (E). The calcium signal, but not the Actin signal, demonstrates significantly higher TE than any of the randomized datasets. The real Actin signal data demonstrates significantly less TE than the randomized Actin does with itself. The real calcium signal also demonstrates significantly greater TE than the Actin signal. *** = p> 0.0001, Mann-Whitney test.

Figure 10: **Information metrics between the calcium and Actin signals**.

MI between the Actin signal of nearby ROIs, between the calcium signal of nearby ROI, and between Actin and calcium signal of nearby ROIs is significantly higher than MI between the randomized Actin signal and calcium or the randomized calcium signal to Actin. The highest MI is observed between nearby ROIs within the calcium signal, with MI between nearby ROIs for the Actin signal being significantly higher than MI between the two signals (A). Within nearby ROIs the both the Actin and calcium signals have significant TE to the calcium signal compared with randomized controls, with the Actin and calcium TE to calcium being statistically insignificant. Neither has significant TE to the Actin signal (B). Within a single ROI the Actin and calcium signals have significant MI, but while the calcium has significant TE to calcium the reverse is not true (C). From these data we propose a model in which Actin establishes boundaries through which calcium signals flow (D). *** = p> 0.0001, Mann-Whitney test.