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Interlinked switch circuits of biological intelligence

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Eukaryotic cells learn and adapt via unknown network architectures. Recent work demonstrated a circuit of two GTPases used by cells to overcome growth factor scarcity, encouraging our view that artificial and biological intelligence share strikingly similar design principles and that cells function as deep reinforcement learning (RL) agents in uncertain environments.

Cells are intelligent machines

The era of single-cell biology has revealed that individual cells in multicellular organisms can sense, decide, and act purposefully. In defining the right decision or action under an evolving environment, cells do not act as subservient robots [1]; instead, they fulfill core properties the technology world equates to 'intelligence' and 'autonomy.' Although intelligent behavior of cells responding to perturbations has been described by many groups, the composition, properties, and architecture of the communication networks within the cells that support intelligent behavior have remained elusive.

Decoding the signaling network that supports intelligent cellular behavior: decades in the making

Prior large-scale efforts to decode the eukaryotic cell's communication network [2] solved many important pieces of the puzzle, such as the importance of the molecular switches [3], in particular the heterotrimeric (tG) [4] and monomeric (mG) [5,6] GTPases of the Ras superfamily. Switches are perfect regulatory modules in any network because they can implement local goals that are, to some degree, self-maintained and self-controlled by their dedicated modulators, that is, guanine nucleotide exchange factors (GEFs; that turn them 'ON') and GTPase-activating proteins (GAPs; that turn them 'OFF'). Relative independence from the rest of the system enables each module to achieve its goals despite changing conditions, contexts, and locations. Consistently, a long-standing tenet was that these two GTPase modules functioned independently of each other: tGs were believed to primarily gate incoming signals from the outside (i.e., sensing), and mGs were believed to primarily coordinate membrane and cytoskeletal remodeling (i.e., responding).

This changed recently with the report of the existence, composition [7], and functional consequences [8] of a Golgi-localized circuit composed of both mG and tG modules. These studies, conducted largely on eukaryotic cells of epithelial origin, describe the conditional self-assembly of the circuit only when cells sense scarcity of growth factors [8] (Box 1). The circuit enables cells to respond by initiating secretion-coupled autocrine signaling, which translates into self-sustenance, survival, and homeostasis [8]. Omics-based approaches pinpointed that such self-sufficiency can be achieved specifically within the epidermal growth factor/epidermal growth factor receptor (EGF/EGFR) signaling pathway (Figure 1A), and studies using recombinant EGF confirmed that activation of the EGF/EGFR pathway at the plasma membrane (PM) is sufficient to activate each component within the Golgi-localized circuitry (Figure 1B) [8].

It is noteworthy that, although the components [9] (Figure 1B) of these self-assembled EGF/EGFR autocrine networks for the maintenance of homeostasis in multicellular epithelial monolayers had been delineated, what couples sensing of EGF to secretion of EGF remained unknown ('black box'; Figure 1B). By virtue of its ability to enable such coupling, the recently defined Golgi-localized circuit appears as a credible candidate for this 'black box.'

The circuit, by design, represents a complex higher-level module that is assembled when two simpler low-level modules (the tG and mG switches) are coupled hierarchically to complete a feedback loop pattern (Box 1). The modules coregulate each other (Figure 1C), and the loop pattern completes each time when mG is turned 'ON.' Completion of the loop circuitry is facilitated by a multimodular scaffold, *G*α-*i*nteracting *v*esicle-associated (GIV) protein (also known as Girdin), which not only directly binds the two GTPases and initiates the loop (Figure 1C,D) but also scaffolds the GAP for mG (ArfGAP2/3) that completes the loop. Thus, this circuit exemplifies three key properties of a network, that is, modularity, hierarchy, and pattern completion, all properties of living systems that enable components to work together toward higher-level goals [10].

Cellular versus artificial intelligence

We believe the ability of the cell to use its Golgi-localized circuit to decide how to act in uncertain environments resembles the mathematical framework of an adaptive learning system, such as a deep reinforcement learning (RL) agent (Figure 1E, F). RL is a type of machine learning technique [11] that enables an agent to learn in an interactive environment by trial and error and mimics reward (or penalty)-based learning and decision-making in humans and other animals. The agent selects an appropriate strategy in relation to its goals, which dictates its actions. RL helps the agent to learn the most optimal policy that maximizes the 'reward function,' which is an incentive mechanism by which the environment informs the

Box 1. A circuitry with six features

Like any intelligent system, our cells learn by (inter)acting with and/or on their environment and adjusting their actions to meet goals. Recently, a molecular circuitry of two interconnected switches has been discovered [8] (see Figure 1B in the main text), which provides the first glimpse into the architecture of the signaling network that supports intelligent behavior of cells.

The notable features of this circuitry are as follows:

(i) Modularity: it is composed of two GTPase modules, each capable of self-maintenance and self-control.
(ii) Hierarchy: one GTPase module combines with the other to form a sophisticated high-level module that allows mutual coregulation.
(iii) Pattern completion: closed-loop control ensures that activation of the first module activates the second module, which in turn inactivates the first module.
(iv) Purposeful: the circuit is self-assembled only when epithelial cells sense scarcity of growth factors and must secrete them to survive and achieve homeostasis.
(v) Proportionate: its design principles ensure that sensing (dose of stimuli) and secreting (response) are aligned to achieve a self-sustained autocrine loop.
(vi) Supports learning and adaptation: by virtue of its ability to train a cell to sense what it secretes as a 'reward' that incentivizes further secretion, the circuit supports adaptive or reinforcement learning (RL) (Figure 1D).

These first glimpses into the architecture of an RL circuit in cells point to an exciting possibility of finding other examples.

agent what helps/hurts using reward/punishment (Figure 1G). The agent must strive to maximize the total reward over time, influencing it exclusively through its own actions (Figure 1H,I). We hypothesize that the epithelial cell (agent) responds to growth factor scarcity (environmental uncertainty) by assembling a circuitry that optimizes growth factor secretion (actions) (Figure 1H, top). Because of the cell's action, the changing environment results in a 'reward' (of secretion-coupled growth signaling), and the cell meets its goal (i.e., survive, proliferate, maintain homeostasis) (Figure 1H, bottom). Thus, biological and artificial intelligence both exemplify agents interacting with and learning within an uncertain environment, and the circuit provides a glimpse of the network that supports such behavior beyond just superficial mimicry or analogies.

Cellular intelligence in health and disease

As for its physiological relevance, this circuitry is likely to impact numerous cellular functions that require the secretory pathway, for example, composition, function, and repair of the PM, control of cell size, and the regulation of the extracellular matrix. The circuit's GTPase modules could represent points of vulnerability because their targeted disruption with toxins (i.e., Arf1 with the fungal toxin, brefeldin A, or Gαi with the exotoxin produced by *Bordetella pertussis*) causes cell death [8].

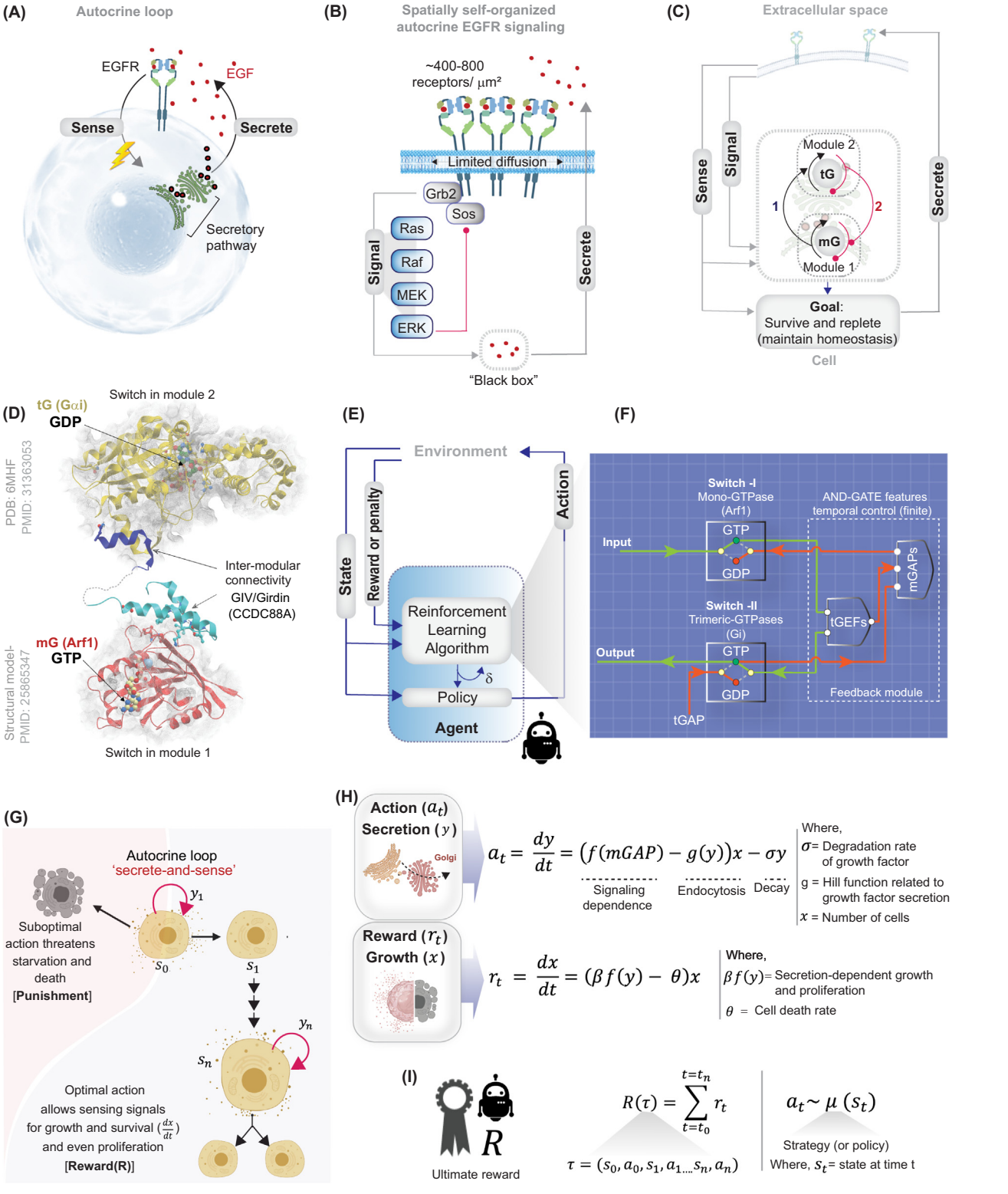
As for what cells 'learn' in the presence of this circuit, it has recently been reported [12] that the circuit supports a 32-gene signature (which includes *EGF*) that is uniquely induced in tumor cells just prior to shedding from the primary tumor and entering the systemic circulation. The signature predicted the metastatic potential of circulating tumor cells (CTCs). Because CTCs en route to metastatic spread represent the riskiest phase in the life of a cancer cell, which lasts ~10 min and is deprived of biologically available EGF [12], the circuit may support evolvability that is far more rapid than the slow Darwinian pace at the primary or metastatic sites.

Concluding remarks

Although we believe the Golgi-localized circuit offers a glimpse into how epithelial cells learn, other cell types may use different circuitry to serve vastly different 'goals.' Because mGs are unique to organelles [6] and tGs are not, it is possible that the circuit(s) repeat(s) elsewhere in cells to make those organelles responsive to the changing environment. To determine if such is the case, there are a few immediately actionable tasks of high priority. On the experimental side of things, because the 'nidus' of the multiscale feedback architecture, that is, the coupled-switch circuit, has emerged (Figure 1F), an easy starting point would be to find examples of such coupling elsewhere within the cell.

On the systems side of things, it is vital to explore if this GTPase circuitry provides robustness in the setting of fluctuating signals/noise, can be converted into oscillators by tuning the cofactor concentrations that catalyze the activity of the cascades, or is much closer to the ideal all-or-none switchlike behavior, all important and emergent properties of network motifs that were observed during simulations on a thermodynamically consistent model for interconnected GTPases [13]. It is possible that the closed-loop circuitry can precisely tune the collective switching of mGTPases along the endomembrane surface (as 'waves') and throughout the entire network, simply by being able to inactivate the Arf1 mGTPase, as shown recently in the case of the Rab mGTPase network [14].

On the computational side of things, quantitative theories are required, much like what led to our understanding of the dopamine-based RL in the brain, which is regarded as one of the greatest successes of computational neuroscience [15]. It is unclear if the circuit, and the 'learning' that it supports, enables a cell to optimize a sequence of actions based on its ability to predict the total reward expected over the future; such ability, also called temporal difference learning [11], is a central feature



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of RL and could be tested in the laboratory by fitting the model to experimental output(s).

In conclusion, the network architecture that supports biological intelligence is expected to help understand the process and products of evolution and anticipate how intrinsic (disease-causing mutations) or extrinsic (our exposome, i.e., microbes, diets, toxins, chemicals, etc.) threats may exploit or corrupt the network. It is also likely that biological intelligence, which has been perfected over billions of years of evolution, could transform the design of artificial intelligence systems.

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Declaration of interests

The authors have no interests to declare.

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References

1. Ford, B.J. (2010) The secret power of the single cell. *New Sci.* 206, 26–27
2. Gilman, A.G. *et al.* (2002) Overview of the Alliance for Cellular Signaling. *Nature* 420, 703–706
3. Bourne, H.R. *et al.* (1990) The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348, 125–132
4. Kleuss, C. *et al.* (1994) Mechanism of GTP hydrolysis by G-protein alpha subunits. *Proc. Natl. Acad. Sci. U. S. A.* 91, 9828–9831
5. Karnoub, A.E. and Weinberg, R.A. (2008) Ras oncogenes: split personalities. *Nat. Rev. Mol. Cell Biol.* 9, 517–531
6. Behnia, R. and Munro, S. (2005) Organelle identity and the signposts for membrane traffic. *Nature* 438, 597–604
7. Lo, I.C. *et al.* (2015) Activation of Gαi at the Golgi by GIV/Girdin imposes finiteness in Arf1 signaling. *Dev. Cell* 33, 189–203
8. Qiao, L. *et al.* (2023) A circuit for secretion-coupled cellular autonomy in multicellular eukaryotic cells. *Mol. Syst. Biol.* 19, e11127
9. Maly, I.V. *et al.* (2004) Self-organization of polarized cell signaling via autocrine circuits: computational model analysis. *Biophys. J.* 86, 10–22
10. Yuste, R. and Levin, M. (2022) New clues about the origins of biological intelligence. *Sci. Am.* 33, 24
11. Sutton, R.S. and Barto, A.G. (1998) *Reinforcement Learning – An Introduction (Adaptive Computation and Machine Learning)*, MIT Press
12. Sinha, S. *et al.* (2024) Growth signaling autonomy in circulating tumor cells aids metastatic seeding. *PNAS Nexus* 3, gae014
13. Ehrmann, A. *et al.* (2019) Interlinked GTPase cascades provide a motif for both robust switches and oscillators. *J. R. Soc. Interface* 16, 20190198
14. Bezeljak, U. *et al.* (2020) Stochastic activation and bistability in a Rab GTPase regulatory network. *Proc. Natl. Acad. Sci. U. S. A.* 117, 6540–6549
15. Schultz, W. *et al.* (1997) A neural substrate of prediction and reward. *Science* 275, 1593–1599

Figure 1. Cells learn and adapt using self-assembled multiscale networks. (A) An autocrine loop that is triggered exclusively when exogenous ligands are scarce generates self-sufficient epidermal growth factor receptor (EGFR)/ErbB signals [7,8]. (B) The components of a self-organized autocrine network for EGFR signaling described previously [9]. Ligand secretion (positive feedback) determines the spatial range of the extracellular signaling, receptor clustering, and the range of intracellular ERK activation to secrete new ligand via unknown processes ('black box'). (C) The components of a Golgi-localized circuitry that is composed of two modules: (i) the mGTPase Arf1 (mG) and (ii) the tGTPase $G_{\alpha}i_{\beta\gamma}$ (tG) and their respective guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). This circuit creates another feedback loop on a different scale (time and space), which ensures that cells sense what they secrete to generate autocrine signals and meet their goal. (D) The structural basis for the assembly of a high-level module of two classes of GTPase switches by linker protein GIV [7]. (E) Schematic of the actor-critic architecture of the deep reinforcement learning (RL) agent interacting with the environment. δ denotes a temporal difference error (see I). (F) A circuit diagram of directional signal flow between various components of the presumed RL circuit. (G and H) Schematic (G) summarizes and the equations (H) capture the behavior of a eukaryptic cell as an agent, faced with environmental uncertainty, must take the most optimal 'action (a_t)' to maximize cumulative 'reward (r_t),'. The cell (G) undergoes a series of growth state changes ($s_0..s_n$) as it adapts to scarcity of growth factors in the environment, purely through a series of its own actions ($a_0..a_n$) of secreting these factors and sensing them as rewards through autocrine secrete-and-sense loops [8]. Cumulative rewards of growth allow the cell to divide (ultimate reward R). Equations (H) capture the experimentally validated behavior in (G) [8], where secretion of growth factors ($\frac{dy}{dt}$) using the GTPase circuitry is an action and autocrine signals that support growth ($\frac{dx}{dt}$) are a reward. (I) Equations define the reward function (r_t) for an agent (a cell), which is determined by how impactful the action was to propel the cell from the state (s_{t-1}) to the next state (s_t). Ultimate reward R is a sum of rewards (r_t) from the current state (s_t) to the goal state (s_n). There is a finite number of time steps ($t_0..t_n$) that are required by a cell to reach R. Trajectory (τ) is a sequence of states (s_t) and actions.

$$\tau = (s_0, a_0, s_1, a_{1....}s_n, a_n)$$

Whether an action at any given time (a_t) is appropriate to achieve the next state (s_{t+1}) is determined by the agent's policy or strategy (μ).

$$a_t \sim \mu\,(s_t).$$

The difference between ultimate reward R (i.e., cell can divide) and the current reward r_t (the amount of growth signals the cell can sense at state s_t) is a temporal difference error, annotated as δ in (E).