Digital kinases
A cell model for sensing, integrating and making choices

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Protein kinases mediate most of the signal transduction in eukaryotic cells, controlling important cellular processes. Functioning as sensors and switches, kinases play a critical role in the regulation of cell fate decisions: proliferation, differentiation or death. Cellular sensors must have signaling properties well suited for the processing and propagation of external or internal stimuli that promote irreversible processes. These properties include ultrasensitivity, hysteresis and digital responses. Ultrasensitivity means to produce a very large response to a small increase in stimulus after a threshold is crossed, hysteresis (a form of biochemical memory) means sustained activation when the stimulus has disappeared, and digital is an all-or-none response at a single cell level. These properties are present in JNK, a stress protein kinase that regulates cell death. In a recent article, we have characterized Xenopus AMPK, a stress protein kinase that controls energy levels in the cell, showing that it is regulated similar to the mammalian ortholog. By using Xenopus oocytes we studied the AMPK signaling system and compared to JNK. Our work showed that AMPK is ultrasensitive to an apoptotic stimulus (hyperosmolar sorbitol) but, in contrast to JNK, does not show hysteresis. By single cell analysis we found that the response of AMPK and JNK to hyperosmolar sorbitol is all-or-none (digital) in character, and that initial graded responses of both protein kinases are converted into digital during the critical period of cytochrome c release. We proposed a model to explain the cell death program as integration of multiple digital signals from stress sensors, that now I extend to a more general model for sensing, integrating and making choices in the cell and the organism.

More that 20 years ago, Tony Hunter proposed that protein kinases may be used in cellular regulatory circuits in a fashion analogous to transistors or chips in computers. In electronic circuits transistors are used either as simple on/off switches or as amplifiers for an electric current. Transistors commonly have two inputs, which regulate current flow and gain, and a single output. Protein kinases have switching properties. For instance, Ca2+/calmodulin-dependent protein kinase II (CaMKII) has the properties of a molecular switch, becoming independent of the Ca2+ signal following Ca2+-stimulated autophosphorylation. The elegant works of James E. Ferrell et al. showed that phosphorylation of MAPK or JNK can yield ultrasensitivity (reflected in a stimulus/response curve with a very steep upstroke), and that ultrasensitive systems embedded in a positive feedback loop have the potential to exhibit bistable behavior, switching between discrete stable steady states without being able to rest in intermediate states. The analogy with a transistor is particularly striking, as well pointed by D. Grahame Hardie years ago. Given the switching properties of these enzymes one can build a sophisticated multidimensional regulatory network with exquisite selectivity and sensitivities using protein kinases at the nodes. We can consider living cells as information-processing systems.
devices. The input information received in the cell from the internal and external environment would be integrated and processed into output responses or cellular choices (to proliferate, to differentiate or to die). Thus, a cellular circuitry based on protein kinases would integrate the variety of different inputs into a common output or cellular decision.

The most irreversible decision for a cell is to commit suicide. The cell must decide, in the face of noxious environmental stimuli, whether to survive and repair the damage or to die by apoptosis. Kinases may play an important role in the “sensing” phase of cell death, ultimately determining cell fate. Some kinases are directly activated, others are turned off, and still others are indirectly activated when cleaved by caspases. Death could be determined by an indirect activation when cleaved by caspases Z-VAD.fmk, and AMPK and JNK responses were not completely correlated with cytochrome c release at the level of individual oocytes.

We can interpret these results in two ways: (1) AMPK and JNK are not involved in cytochrome c release and another unknown factor is responsible for cell death; or (2) AMPK or JNK digital responses are not sufficient to induce apoptosis but the combination of digital responses from different stress sensors can engage the cell death program (see Fig. 1). I prefer the second hypothesis for several reasons: (1) Based in the literature, it would be very surprising that the rapid activation of AMPK and JNK (just a few minutes after hyperosmolar sorbitol treatment) will not have any effect in the regulation of cell death (another question is how: preventing or inducing), (2) When AMPK and JNK are “digitally” activated in the same cell there is a high probability to find cytochrome c release and caspase activation, and (3) Integration of different signals from different sensors seems logical in such an important and irreversible decision as cell death.
The number of oscillations depends on the radiation dose. A model was proposed where ATM kinase, which is a sensor of double strand breaks in the DNA, acts as a switch activating the p53—Mdm2 oscillator module in a digital way. Interestingly, the authors speculate that pro-apoptotic p53 target genes will be gradually integrated over multiple cycles of p53 pulses until they reach a certain threshold value that will activate apoptosis.

If we take again the analogy of cells as information devices and of protein kinases as transistors or chips in computers, we can imagine networks of kinases that generate digital information to "run" different programs (like the cell death program) (Fig. 2). Therefore, a general model for sensing, integrating and making choices, based in the generation of digital responses, may be applied to different biological processes. However, we cannot discard cellular programs based in analog signals, depending of the stimulus. In fact, analog and digital programs could be rewired. For instance, MAP kinase modules are tightly controlled by positive and negative feedback loops, as well as a variety feed-forward loops. Different feedback control systems can theoretically allow the same module to generate different signal outputs, including graded (analog) or digital responses. The number of oscillations depends on the radiation dose. A model was proposed where ATM kinase, which is a sensor of double strand breaks in the DNA, acts as a switch activating the p53—Mdm2 oscillator module in a digital way. Interestingly, the authors speculate that pro-apoptotic p53 target genes will be gradually integrated over multiple cycles of p53 pulses until they reach a certain threshold value that will activate apoptosis.

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activation in PC-12 cells (inducing cell proliferation), whereas NGF treatment produced a digital ERK output (required for differentiation). The authors found that PKC phosphorylation of Raf kinase inhibitory protein (RKIP) reconfigures the MAPK module from an analog system to digital one by a positive feedback loop mechanism, thereby directing opposing cell fate decisions.14 Tian et al. have shown that activation of the MAPK module through Ras nanoclusters in the cell membrane generates high-fidelity analog output functioning as an analog-digital-analog converter.15 Recently, it has been described that Ras activation in lymphoid cells is characterized by digital signaling and hysteresis as a consequence of a positive feedback loop.16 In principle, it could be possible to develop algorithms to predict outputs from specific inputs (signals) if we know all the pathways involved and the basic properties of the nodes (protein kinases) in the network. Can we extend this reasoning to the whole organism? Or at least, can we extend it to an important organ as the brain?

The majority of protein kinases are expressed in the brain. It seems possible that many of them show digital responses to physiological stimuli. Most studies of signal transduction use saturating doses of the stimuli and one can question whether the responses obtained are physiologically relevant. We observed digital responses of AMPK and JNK in single cells (oocytes) at intermediate doses of sorbitol. I propose that physiological concentrations of different stimuli in the brain can generate digital responses of protein kinases in neurons.

Now, I will consider a fundamental process in the brain: memory. Long-term potentiation (LTP) is an activity-dependent strengthening of synapses that is though to underlie memory storage. Interestingly, individual synapses appear to have all-or-none potentiation with different thresholds.17 Therefore, it seems that synaptic memories at hippocampus are encoded in a digital manner. LTP is regulated by several protein kinases, including cAMP-dependent protein kinase (PKA), PKC, MAPK, CaMKII18 and the atypical PKC isozyme protein kinase Mzeta (PKMζ).19 Importantly, persistent protein kinase activity exists in LTP for CaMKII20 and PKMζ.21 A model of long term memory has been proposed based in the molecular switch properties of of CaMKII.22 CaMKII is necessary for long-term potentiation (LTP) in the hippocampus. The kinase can be activated to different degrees and is able to function as a frequency detector. CaMKII acts as a bistable switch in the postsynaptic density, turning on when a threshold number of kinase sites are phosphorylated. The on state of the switch can last for very long periods because the kinase acts faster that the phosphatase (reviewed in ref. 23). Indeed, PKMζ, acting as a constitutive kinase, is necessary and sufficient for LTP maintenance23 and is required for several types of long-term memories.24

Some years ago, a digital model was proposed for the process of memory and retrieval of the stored information (recall). It was postulated that spike trains (brief sequence of action potentials) generated by neurons are digital signals representing binary numbers (coded information) and are used by the brain to perform internal calculations.25 Cesare Marchetti proposed that a spike train could be the way that a neuronal event becomes digitized, so that the memory of this event can be reactivated by a number carried by a spike train roaming the brain.26 The same author also proposes that a spike train representing a digital number sets CaMKII into one of its 10² potential states (“freezing the information”), and later a spike train carrying the same number will reactivate it in terms of stimulating calmodulin to liberate Ca²⁺. A recall requires the simultaneous switching on of thousands of synapses by a signal of some sort that travels through the brain. Who and how starts the chain of events leading to a recall? Marchetti reasons that a single cell may hold the code of a single memory in the form of one or more molecules of coded CaMKII and would be able to initiate the spike train that will open the circuit.

A model for long term memory based in one or two protein kinases (CaMKII and PKMζ) is attractive, but taking into account that many protein kinases show digital responses we should consider an scenario where multiple digital signals are integrated: first at the level of single cells generating an output, and later as a net of cells that would generate a recall, or even a new idea as a result of mixed outputs. If this becomes true, to analyze the signaling events in single cells and the networks of digital responses generated in the brain could help to understand how we think and learn, and eventually how we take decisions.

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