Bistability in the JNK cascade

Christoph P. Bagowski and James E. Ferrell, Jr.

Background: Important signaling properties, like adaptation, oscillations, and bistability, can emerge at the level of relatively simple systems of signaling proteins. Here, we have examined the quantitative properties of one well-studied signaling system, the JNK cascade. We experimentally assessed the response of JNK to a physiological stimulus (progesterone) and a pathological stress (hyperosmolar sorbitol) in *Xenopus laevis* oocytes, a cell type that is well-suited to the quantitative analysis of cell signaling. Our aim was to determine whether JNK responses are graded (Michaelian) in character; ultrasensitive in character, resembling the responses of cooperative enzymes; or bistable and all-or-none in character.

Results: The responses of JNK to both progesterone and sorbitol were found to be essentially all-or-none. Individual oocytes had either very high or very low JNK activities, with few oocytes possessing intermediate levels of JNK activity. Moreover, JNK activation was autocatalytic, indicating that the JNK cascade is either embedded in or downstream of a positive feedback loop. JNK also exhibited hysteresis, a form of biochemical memory, in its response to sorbitol. These findings indicate that the JNK cascade is part of a bistable signaling system in oocytes.

Conclusions: In *Xenopus* oocytes, JNK responds to physiological and pathological stimuli in an all-or-none manner. The JNK response shows all the hallmarks of a bistable response, including strong positive feedback and hysteresis. Bistability is a recurring theme in the biochemistry of oocyte maturation and early embryogenesis; the Mos/MEK/p42 MAPK cascade also exhibits bistable responses, and the Cdc2/cyclin B system is hypothesized to be bistable as well. However, the mechanisms underpinning the positive feedback and bistability in the three cases are different, suggesting that evolution has repeatedly converged upon bistability as a way of producing digital responses.

Address: Division of Chemical Biology, Stanford University School of Medicine, Stanford, California 94305-5174, USA.

Correspondence: James E. Ferrell, Jr.
E-mail: james.ferrell@stanford.edu

Received: **6 June 2001**
Accepted: **28 June 2001**

Published: **7 August 2001**

Current Biology 2001, 11:1176–1182

0960-9822/01/$ – see front matter

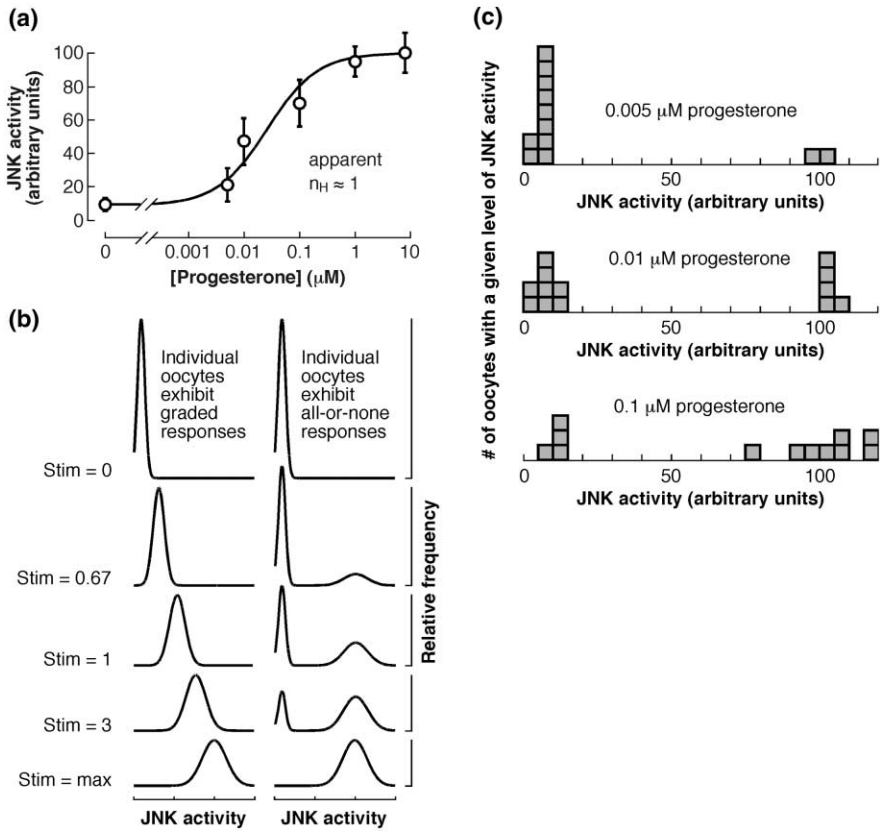
Background

The Jun N-terminal kinases (JNKs) or stress-activated protein kinases (SAPKs) are a family of evolutionarily conserved protein kinases implicated in stress responses and apoptosis [1–4]. Fibroblasts from mouse embryos with knockouts in the broadly expressed *jnk1* and *jnk2* genes fail to carry out apoptosis in response to some normally apoptotic stimuli [5], and neurons from mice with knockouts in the more selectively expressed *jnk3* gene have defects in excitotoxin-induced neuronal cell death [6]. Like all members of the MAP kinase family, JNKs are components of an evolutionarily conserved, three-tiered kinase cascade. An upstream MAP kinase kinase kinase (or JNK kinase kinase) phosphorylates and activates the relevant MAP kinase kinases (MKK4 and MKK7), which in turn phosphorylate and activate the JNKs.

It is becoming increasingly appreciated that important biochemical behaviors can emerge at the level of small signaling modules [7–12]. For example, a protein kinase cascade like the JNK cascade could, in principle, function as a sensitivity amplifier, converting graded inputs into more switch-like, ultrasensitive outputs, allowing the cascade to filter out noise and yet still respond decisively to supra-threshold stimuli [13–16]. If the cascade were embedded in a positive feedback loop (which has not previously been demonstrated for the JNK cascade), the system would have the potential to exhibit bistable behavior, switching between discrete stable steady states without being able to rest in intermediate states [17, 18]. Since JNK is implicated in apoptosis, and apoptosis is an all-or-none biological event, it might be appropriate for the JNK cascade to exhibit either an ultrasensitive or a bistable response. Either type of response would effectively mean that JNK relayed a digital, all-or-none signal to downstream effectors of apoptosis. Alternatively, the JNK cascade might transmit graded, analog information about how stressful a cell's environment is, with the life-or-death decision made downstream.

Here, we have addressed the question of whether JNK responses are graded or all-or-none in character. The ex-

Figure 1



JNK activation in progesterone-treated oocytes. Oocytes were incubated with various concentrations of progesterone in oocyte Ringer's solution (OR2) for 12 hr. Oocytes were lysed, and the lysates were subjected to GST-Jun precipitation followed by an in vitro kinase assay. **(a)** JNK activities in pools of oocytes. Each point represents a group of 11 or 12 oocytes. Data are shown as averages plus or minus one standard error of the mean. The data are well fitted by a Michaelian stimulus-response curve with a Hill coefficient (n_H) of 1. **(b)** Expected distributions of oocytes among various levels of JNK activity. On the left, we assumed that the individual oocytes shift from an off-state with 8 ± 4 units of JNK activity, as observed for the untreated oocytes in (a), to an on-state with 100 ± 20 units of JNK activity, as observed for the oocytes treated with 10 μM progesterone, in a graded manner. On the right, we assumed that the individual oocytes shift from the off-state to an on-state in a switch-like, all-or-none manner. In both cases, the level of stimulus (Stim) is expressed in multiples of the EC50. At intermediate stimulus levels, the graded model predicts a unimodal distribution of oocytes with intermediate levels of JNK activity, whereas the all-or-none model predicts a bimodal distribution of oocytes. Modeled responses were calculated with Mathematica 2.2.2. **(c)** Measured JNK activities of individual oocytes. Each box represents one individual oocyte. For simplicity, only the oocytes incubated with intermediate concentrations of progesterone (0.005–0.1 μM) are shown.

perimental system we chose for these studies is the *Xenopus* oocyte. By virtue of its large size (an oocyte has about 250,000 times as much cytoplasm as a typical somatic cell), the oocyte allows signal transduction events to be biochemically assessed at the level of the individual cell, which can be important for discerning the character of a signaling response [17]. Recent work has established that *Xenopus* oocytes possess a JNK protein, that *Xenopus* JNK becomes activated in response to progesterone just prior to germinal vesicle breakdown in maturing *Xenopus* oocytes, and that JNK then remains constitutively active until the early gastrula stage of embryogenesis [19]. JNK can also be activated in immature oocytes by exposing the oocytes to hyperosmolar stresses [19]. The demonstration that *Xenopus* JNK can be both pathologically and physiologically activated and the availability of powerful techniques for the quantitative analysis of signal transduction in oocytes make this cell system particularly suitable for the present studies.

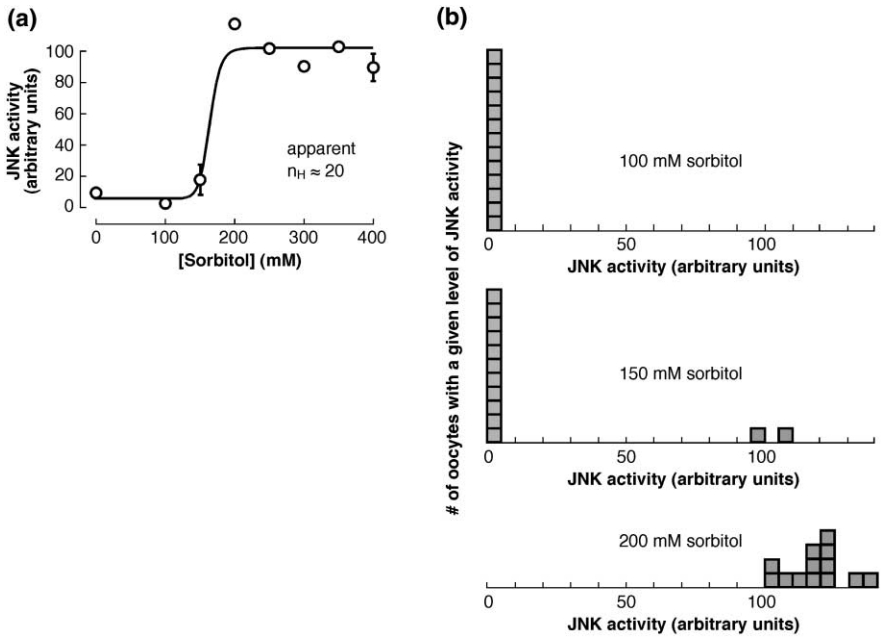
Here, we show that the response of JNK to both progesterone and sorbitol is essentially all-or-none at the level of the individual oocyte. JNK switches rapidly between the off-state and the on-state and exhibits hysteresis in its response to increasing and decreasing stimuli. Microinjection studies show that JNK is either embedded in or downstream of a strong positive feedback loop. Thus, in oocytes, JNK exhibits a bistable response to upstream stimuli, which can be mechanistically attributed to the presence of positive feedback. The oocyte's Mos/MEK/p42 MAPK cascade also exhibits bistable responses [17], suggesting that this sort of digital, bistable response is an important theme in cell signaling.

Results

Graded response of JNK to progesterone in pools of oocytes

We incubated oocytes with various concentrations of progesterone and allowed the oocytes to approach a steady state, in which JNK activity was unchanging with respect to time. We then lysed the oocytes, precipitated the JNK with GST-Jun beads, and subjected the precipitated JNK to an in vitro kinase assay. In pools of oocytes, JNK activity was found to be a graded function of the progesterone

Figure 2



JNK activation in sorbitol-treated oocytes. Oocytes were incubated for 4 hr in oocyte Ringer's solution (OR2) supplemented with various concentrations of sorbitol. Oocytes were lysed, and the lysates were subjected to GST-Jun precipitation and subsequent kinase assay. **(a)** JNK activities in pools of oocytes. Each point represents a group of 13 oocytes. Data are shown as averages plus or minus one standard error of the mean. The data are well fitted by an ultrasensitive Hill equation curve with a Hill coefficient (n_H) of 20. **(b)** Measured JNK activities in individual oocytes. Each box represents one individual oocyte. For simplicity, only the oocytes incubated with 100, 150, and 200 mM sorbitol are shown.

concentration, well-approximated by a Hill function with a Hill coefficient of 1 (Figure 1a). Thus, at the level of a population of oocytes, the response of JNK to progesterone is graded.

All-or-none response of JNK to progesterone in individual oocytes

The graded JNK response could mean either that individual oocytes exhibited graded responses (Figure 1b, left) or that individual oocytes exhibited switch-like responses but had widely varying thresholds for tripping the switch (Figure 1b, right). These two possibilities were distinguished by examining JNK activities in individual oocytes incubated with intermediate concentrations of progesterone. If the oocytes were all found to have intermediate levels of JNK activity (Figure 1b, left), it would mean that the response of an individual oocyte was graded. However, if the individual oocytes had high or low JNK activities, but not intermediate JNK activities (Figure 1b, right), it would mean that the individual oocytes' responses were switch-like. As shown in Figure 1c, the individual oocytes were found to adopt a bimodal distribution, with very few oocytes exhibiting intermediate levels of JNK activity. Thus, at the level of a population of oocytes, the JNK response is graded, but at the level of an individual oocyte, the response of JNK to progesterone is switch-like.

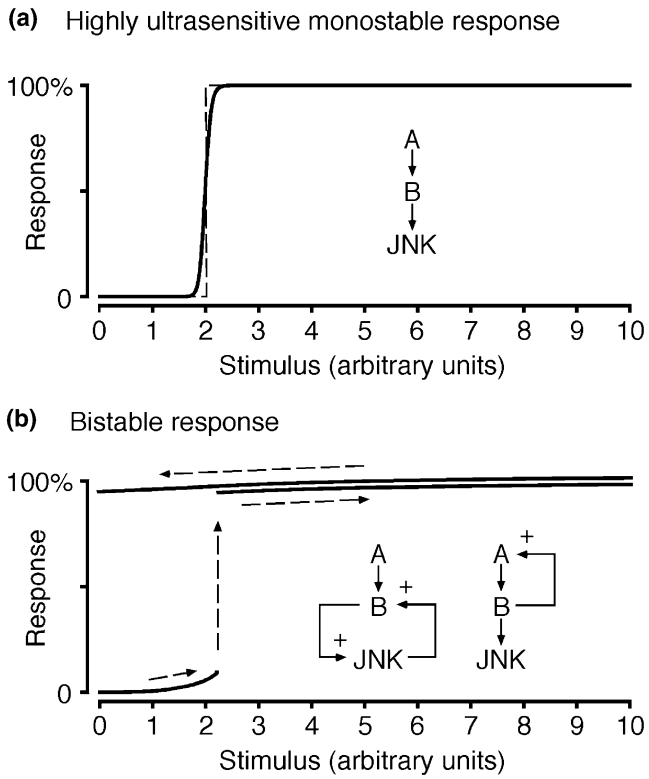
All-or-none response of JNK to sorbitol in individual oocytes

The Mos/MEK/p42 MAPK cascade in oocytes can bring about activation of JNK [19], and the response of Mos/MEK/p42 MAPK to progesterone is essentially all-or-none [17]. Thus, it seemed possible that the switch-like response of JNK to progesterone was imposed upon it by the Mos/MEK/p42 MAPK cascade, rather than being intrinsic to the JNK cascade. We therefore set out to determine whether the response of JNK to stimuli that do not activate Mos/MEK/p42 MAPK would be graded rather than switch-like. We incubated oocytes with various concentrations of hyperosmolar sorbitol, which activates JNK, but not p42 MAPK [19], allowed the oocytes to approach a steady state, and assessed JNK activity. The response of pools of oocytes to sorbitol was ultrasensitive (Figure 2a). The stimulus/response curve had a very steep upstroke and was well-approximated by a Hill function with a remarkably high Hill coefficient of 20 (Figure 2a), indicating that the JNK response to sorbitol is highly switch-like and there is little oocyte-to-oocyte variability in the threshold for the response. Moreover, the oocytes' individual responses were even more switch-like than their pooled responses. The apparently intermediate response of the pool of oocytes to 150 mM sorbitol (Figure 2a) was found to represent a bimodal distribution of individual oocytes: 11 oocytes with inactive JNK, and 2 oocytes with fully active JNK. Thus, the response of JNK to hyperosmolar stress, like the response of JNK to progesterone, was essentially all-or-none at the level of the individual oocyte.

Hysteresis in the JNK response

All-or-none responses can, in principle, arise either from an ultrasensitive, monostable signaling system with a very high Hill coefficient or from a bistable signaling system.

Figure 3



Two types of mechanisms that can produce an all-or-none response. **(a)** A monostable, but highly ultrasensitive, response. This sort of response could be produced through cooperativity, through saturation effects (zero-order ultrasensitivity), or through the effects of stoichiometric inhibitors and could be amplified by a linear cascade (like the A → B → JNK cascade shown here). An ultrasensitive response can approach a step function (dashed line). **(b)** A bistable response. This sort of response depends upon the presence of some sort of mechanism for autocatalysis, such as positive feedback; two possible arrangements of the feedback are shown here. A bistable response is discontinous; the system can rest in either of two alternative steady states, but cannot rest in an intermediate state. A bistable system should also possess hysteresis, meaning that the stimulus-response curve obtained when the system is changed from low stimulus to high stimulus will not be identical to that obtained when the system is changed from high stimulus to low stimulus, as indicated by the arrows. If the positive feedback is sufficiently strong, as it is in the model shown here, a bistable system can be irreversible. Modeled responses were calculated with Mathematica 2.2.2.

An ultrasensitive, monostable system behaves like a doorbell switch; it turns on in an all-or-none manner but requires the continuous presence of a stimulus to remain in its on state (Figure 3a). When the stimulus is removed, the system returns to the same "off" state it was in before the stimulus was applied. A bistable system exhibits a qualitatively different type of behavior. A bistable system should exhibit some degree of hysteresis, meaning that, if the activating stimulus is decreased, the system will remain on until the stimulus is well below the level that initially put the system in the "on" state. If the hysteresis is pronounced enough, it can turn into a form of actively maintained irreversibility, with the system remaining on even after the stimulus is lowered to zero (Figure 3b). In this limit, a bistable system acts like a toggle switch.

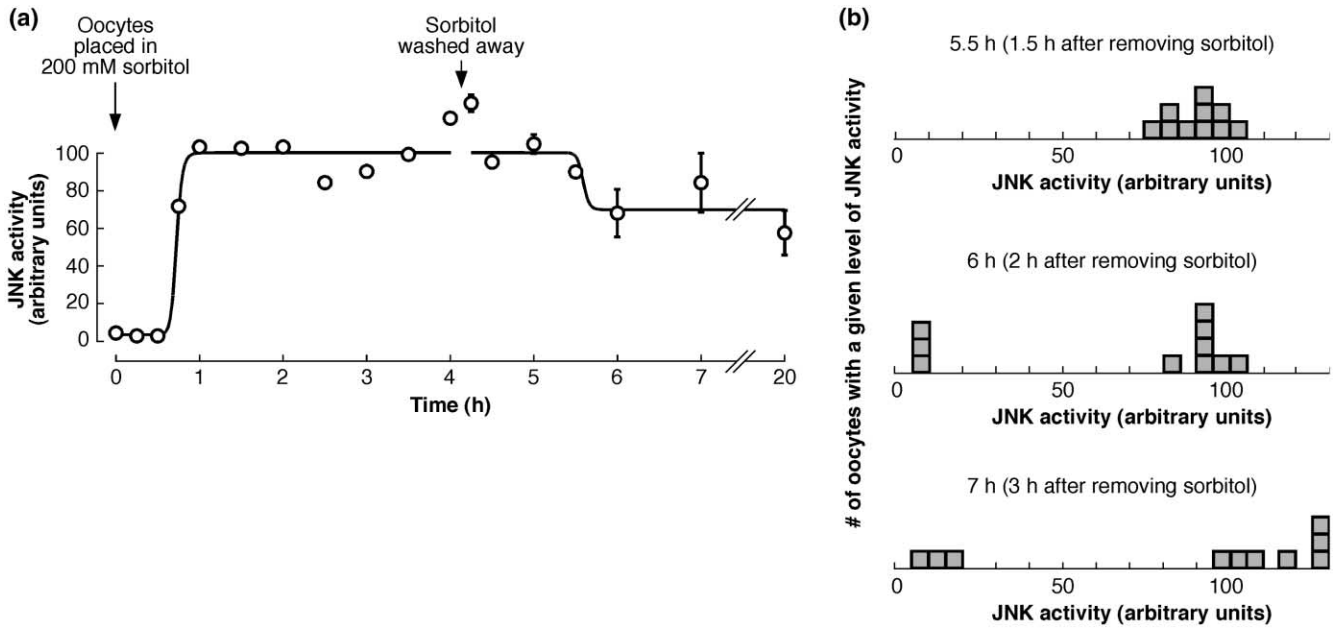
We therefore examined the timing and reversibility of JNK activation in oocytes incubated with hyperosmolar sorbitol (200 mM) and then returned to plain isotonic buffer. The activation of JNK was found to be temporally abrupt; there was no detectable JNK activation during the first 30 min of exposure to sorbitol, and then by 60 min, the activation was maximal (Figure 4a). When the sorbitol was washed away and the oocytes returned to isotonic buffer, there was a small decline in the level of JNK activity (Figure 4a). The analysis of individual oocytes showed that the majority (~70%) of the oocytes retained fully active JNK even as long as 16 hr after the removal of the sorbitol and that the rest of the oocytes had had their JNK activity fall to low levels about 2 hr after the sorbitol was removed (Figure 4b). The abruptness of the changes in JNK activity and the long persistence of JNK activity after the removal of the activating stimulus suggested that the JNK cascade might be a bistable system exhibiting hysteresis.

Autocatalysis in JNK activation

An essential element of any bistable signaling system is a mechanism that provides the system with an autocatalytic character [8, 12, 18, 20–26]. This mechanism can be positive feedback, where a signaling molecule promotes its own activation, or "double-negative" feedback, where two signaling molecules mutually inhibit each other, or a variant of these mechanisms. We therefore examined whether JNK activation in oocytes is autocatalytic.

The experimental approach we took was the sequential cytoplasmic transfer strategy that Masui and Markert used to demonstrate the autocatalytic nature of M phase-promoting factor (MPF) activation [27]. We incubated oocytes with sorbitol, allowed the JNK to become maximally activated, and then obtained cytoplasm from these oocytes (Figure 5a). We injected various dilutions of the cytoplasm into fresh oocytes and then assessed the resulting steady state activity of JNK. We found that the cytoplasm from the sorbitol-treated oocytes did promote full JNK activation in the microinjected oocytes, sorbitol caused the formation of a JNK activation-promoting factor (JPF) in the sorbitol treated oocytes, and that the titer of the JPF activity was between 1:40 and 1:60 (Figure 5a). We then obtained cytoplasm from these oocytes and injected it into a second fresh batch of oocytes. The injected cytoplasm again promoted JNK activation in the recipient oocytes (Figure 5b). Moreover, the titer of the JPF activity was undiminished (Figure 5b), despite the fact that each

Figure 4



The time course of JNK activation and inactivation in sorbitol-treated oocytes. **(a)** Overall responses. The time points from 0 to 4 hr represent groups of 5 oocytes per time point. After 4 hr of incubation with 200 mM sorbitol, the oocytes were washed 3 times in isotonic oocyte Ringer's solution (OR2) and resuspended in OR2. Ten oocytes were collected per time point after the washing. **(b)** Measured JNK activities in individual oocytes. Each box represents one individual oocyte. For simplicity, only the 5.5, 6, and 7 hr time points are shown.

round of microinjection dilutes the cytoplasm by a factor of about ten. Thus, JPF must promote its own activation or production; the JNK cascade in oocytes is autocatalytic.

Discussion

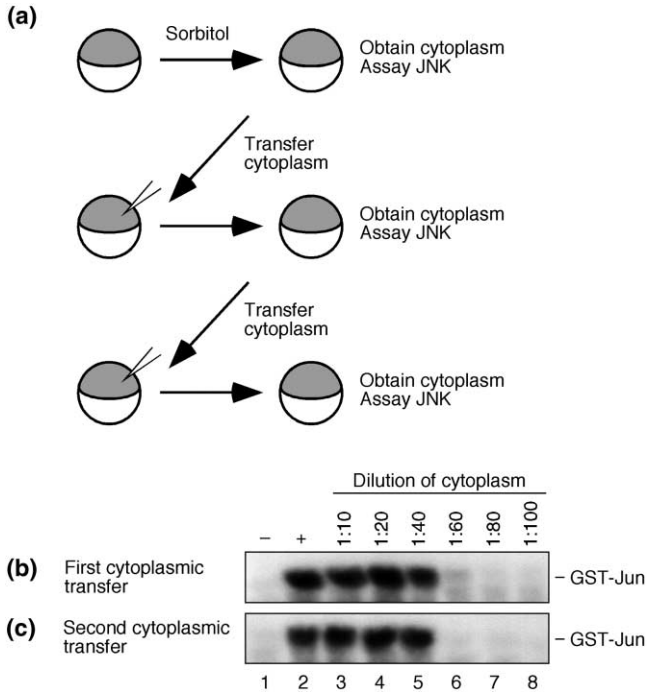
We have taken advantage of the ability to analyze the responses of individual oocytes to examine how the JNK cascade responds to a physiological stimulus (progesterone) and a pathological stress (hyperosmolar sorbitol). We found that JNK activation in oocytes is all-or-none, temporally abrupt, autocatalytic, and persistent for long periods of time after the initiating stimulus is removed. These are all of the hallmarks of a bistable response. This sort of response has long been assumed by theoretical biologists to be of critical importance for cell signaling [9], and, indeed, artificial bistable gene regulatory systems have now been engineered into bacteria [23] and yeast [28]. Nevertheless, there has been little direct experimental evidence for or against bistability in cell signaling. The present results provide strong evidence that the JNK system in oocytes is bistable and that the overall character of the JNK response in oocytes is essentially digital. Thus, in oocytes, the JNK system is not simply a passive pipeline that relays information for downstream signaling elements to interpret; sufficient signal processing takes place at the level of JNK and above to turn a graded input into a decisive, all-or-none response.

Bistable systems have the potential to remain on long after the stimulus that initially triggered its activation has been removed; bistable systems can possess an actively maintained memory of the triggering stimulus. Forty years ago, Monod and Jacob hypothesized that bistable signaling systems might provide cells with the sort of long-term memory required to maintain differentiation long after a differentiation-inducing stimulus is removed [9]. The present work indicates that, in oocytes, JNK activation is one such memory system. JNK activation in oocytes is physiologically triggered by progesterone, but JNK remains active throughout early embryogenesis, long after the initiating progesterone stimulus has ceased [19]. The present results provide a mechanistic explanation for how this long-term activation of JNK is achieved.

JNK is not the only bistable signaling system present in the oocyte. The Mos/MEK1/p42 MAPK cascade appears to be bistable as well [17], and, by virtue of its autocatalytic character, Cdc2-cyclin B may also be part of a bistable system [12, 27, 29, 30]. Thus, bistability and all-or-none signal transduction systems may be common. The emerging picture is that oocyte maturation and early embryogenesis are characterized by, and probably driven by, combinations of essentially digital, all-or-none responses that arise out of interconnected bistable signaling pathways.

However, although the JNK, p42 MAPK, and Cdc2-cyclin

Figure 5



Titration of JNK activation-promoting factor activities after cytoplasmic transfer. JNK activity was monitored by the transfer of ^{32}P from [γ-^{32}P]ATP to GST-Jun after lysing cells and pulling-down JNK on GST-Jun beads. **(a)** A schematic depiction of the serial transfer experiment. **(b)** JNK activities after microinjecting oocytes with various dilutions of cytoplasm from sorbitol-treated oocytes. **(c)** JNK activities after microinjecting oocytes with various dilutions of cytoplasm from the injected oocytes used in (b). Lane 1, untreated oocytes; lane 2, sorbitol-treated oocytes; and lanes 3–8, oocytes injected with various dilutions of cytoplasm. The dilutions indicated in (b) and (c) are final dilutions after microinjection.

B systems all appear to produce bistable responses through positive feedback, the specific mechanisms through which the feedback is produced and the bistability is achieved are all different. In the case of p42 MAPK, the positive feedback is provided by translational regulation: p42 MAPK promotes the translation of its upstream activator Mos and promotes the stabilization of the Mos protein [31–34]. In the case of JNK, JNK activation is unimpaired in cycloheximide-treated oocytes (data not shown), implying that the positive feedback must be post-translational. In the case of Cdc2, both translational and posttranslational feedback may be important: Cdc2 activation stimulates cyclin translation, but Cdc2 can also post-translationally stimulate its activator Cdc25 and inhibit its inhibitors Wee1 and Myt1 [29–31, 35, 36]. Thus, it appears that nature has converged upon the same basic scheme for producing decisive, digital responses again and again, but has done so through a variety of different specific mechanisms. This convergent evolution underscores the likely importance of bistability in cell signaling.

Materials and methods

Xenopus oocytes were defolliculated by collagenase treatment, as described [19]. After treating the oocytes with progesterone or sorbitol in oocyte Ringer's solution (OR2, 82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 5 mM HEPES [pH 7.5]), oocytes were individually collected, frozen, and lysed as described [17, 19]. JNK assays were performed on GST-Jun pull-downs, as described [37] and quantified by phosphorimaging. Care was taken to ensure that JNK activity was linear with respect to time and JNK concentration.

Acknowledgements

We thank A. Ullrich for providing GST-Jun plasmids and Miranda Robertson and members of the Ferrell lab for critical reading of this manuscript. This work was supported by a grant from the National Institutes of Health (GM61276) and a Deutsche Forschungsgemeinschaft Postdoctoral Fellowship.

References

1. Davis RJ: **Signal transduction by the c-Jun N-terminal kinase.** *Biochem Soc Symp* 1999, **64:**1-12.
2. Woodgett JR, Avruch J, Kyriakis J: **The stress activated protein kinase pathway.** *Cancer Surv* 1996, **27:**127-138.
3. Minden A, Karin M: **Regulation and function of the JNK subgroup of MAP kinases.** *Biochim Biophys Acta* 1997, **1333:**F85-104.
4. Davis RJ: **Signal transduction by the JNK group of MAP kinases.** *Cell* 2000, **103:**239-252.
5. Tournier C, Hess P, Yang DD, Xu J, Turner TK, Nimnual A, *et al.*: **Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway.** *Science* 2000, **288:**870-874.
6. Yang DD, Kuan CY, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, *et al.*: **Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene.** *Nature* 1997, **389:**865-870.
7. Bhalla US, Iyengar R: **Emergent properties of networks of biological signaling pathways.** *Science* 1999, **283:**381-387.
8. Smolen P, Baxter DA, Byrne JH: **Frequency selectivity, multistability, and oscillations emerge from models of genetic regulatory systems.** *Am J Physiol* 1998, **274:**C531-542.
9. Monod J, Jacob F: **General conclusions: teleonomic mechanisms in cellular metabolism, growth, and differentiation.** *Cold Spring Harbor Symp Quant Biol* 1961, **26:**389-401.
10. Chock PB, Rhee SG, Stadtman ER: **Interconvertible enzyme cascades in cellular regulation.** *Annu Rev Biochem* 1980, **49:**813-843.
11. Koshland DE Jr, Goldbeter A, Stock JB: **Amplification and adaptation in regulatory and sensory systems.** *Science* 1982, **217:**220-225.
12. Novak B, Tyson JJ: **Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos.** *J Cell Sci* 1993, **106:**1153-1168.
13. Huang C-YF, Ferrell JE Jr.: **Ultrasensitivity in the mitogen-activated protein kinase cascade.** *Proc Natl Acad Sci USA* 1996, **93:**10078-10083.
14. Ferrell JE Jr: **How responses get more switch-like as you move down a protein kinase cascade.** *Trends Biochem Sci* 1997, **22:**288-289.
15. Ferrell JE Jr: **Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs.** *Trends Biochem Sci* 1996, **21:**460-466.
16. Brown GC, Hoek JB, Kholodenko BN: **Why do protein kinase cascades have more than one level?** *Trends Biochem Sci* 1997, **22:**288.
17. Ferrell JE Jr, Machleder EM: **The biochemical basis of an all-or-none cell fate switch in *Xenopus* oocytes.** *Science* 1998, **280:**895-898.
18. Ferrell JE Jr, Xiong W: **Bistability in cell signaling: how to make continuous processes discontinuous, and reversible processes irreversible.** *Chaos* 2001, **11:**227-236.
19. Bagowski CP, Xiong W, Ferrell JE Jr: **c-Jun N-terminal kinase activation in *Xenopus laevis* eggs and embryos: a possible non-genomic role for the JNK signaling pathway.** *J Biol Chem* 2001, **276:**1459-1465.
20. Thron CD: **Bistable biochemical switching and the control of the events of the cell cycle.** *Oncogene* 1997, **15:**317-325.

21. Thron CD: **A model for a bistable biochemical trigger of mitosis.** *Biophys Chem* 1996, **57:**239-251.
22. Laurent M, Kellershohn N: **Multistability: a major means of differentiation and evolution in biological systems.** *Trends Biochem Sci* 1999, **24:**418-422.
23. Gardner TS, Cantor CR, Collins JJ: **Construction of a genetic toggle switch in *Escherichia coli*.** *Nature* 2000, **403:**339-342.
24. Segel LA: **Multiple attractors in immunology: theory and experiment.** *Biophys Chem* 1998, **72:**223-230.
25. Hervagault JF, Canu S: **Bistability and irreversible transitions in a simple substrate cycle.** *J Theor Biol* 1987, **127:**439-449.
26. Guidi GM, Goldbeter A: **Bistability without hysteresis in chemical reaction systems: a theoretical analysis of irreversible transitions between multiple steady states.** *J Phys Chem A* 1997, **101:**9367-9376.
27. Masui Y, Markert CL: **Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes.** *J Exp Zool* 1971, **177:**129-145.
28. Becskei A, Seraphin B, Serrano L: **Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion.** *EMBO J* 2001, **20:**2528-2535.
29. Solomon MJ, Glotzer M, Lee TH, Philippe M, Kirschner MW: **Cyclin activation of $p34^{cdc2}$.** *Cell* 1990, **63:**1013-1024.
30. Hoffmann I, Clarke PR, Marcote MJ, Karsenti E, Draetta G: **Phosphorylation and activation of human cdc25-C by cdc2–cyclin B and its involvement in the self-amplification of MPF at mitosis.** *EMBO J* 1993, **12:**53-63.
31. Howard EL, Charlesworth A, Welk J, MacNicol AM: **The MAP kinase signaling pathway stimulates Mos mRNA cytoplasmic polyadenylation during *Xenopus* oocyte maturation.** *Mol Cell Biol* 1999, **19:**1990-1999.
32. Gotoh Y, Masuyama N, Dell K, Shirakabe K, Nishida E: **Initiation of *Xenopus* oocyte maturation by activation of the mitogen-activated protein kinase cascade.** *J Biol Chem* 1995, **270:**25898-25904.
33. Roy LM, Haccard O, Izumi T, Lattes BG, Lewellyn AL, Maller JL: **Mos proto-oncogene function during oocyte maturation in *Xenopus*.** *Oncogene* 1996, **12:**2203-2211.
34. Matten WT, Copeland TD, Ahn NG, Vande Woude GF: **Positive feedback between MAP kinase and Mos during *Xenopus* oocyte maturation.** *Dev Biol* 1996, **179:**485-492.
35. Mueller PR, Coleman TR, Kumagai A, Dunphy WG: **Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15.** *Science* 1995, **270:**86-90.
36. Mueller PR, Coleman TR, Dunphy WG: **Cell cycle regulation of a *Xenopus* Wee1-like kinase.** *Mol Biol Cell* 1995, **6:**119-134.
37. Hibi M, Lin A, Smeal T, Minden A, Karin M: **Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain.** *Genes Dev* 1993, **7:**2135-2148.