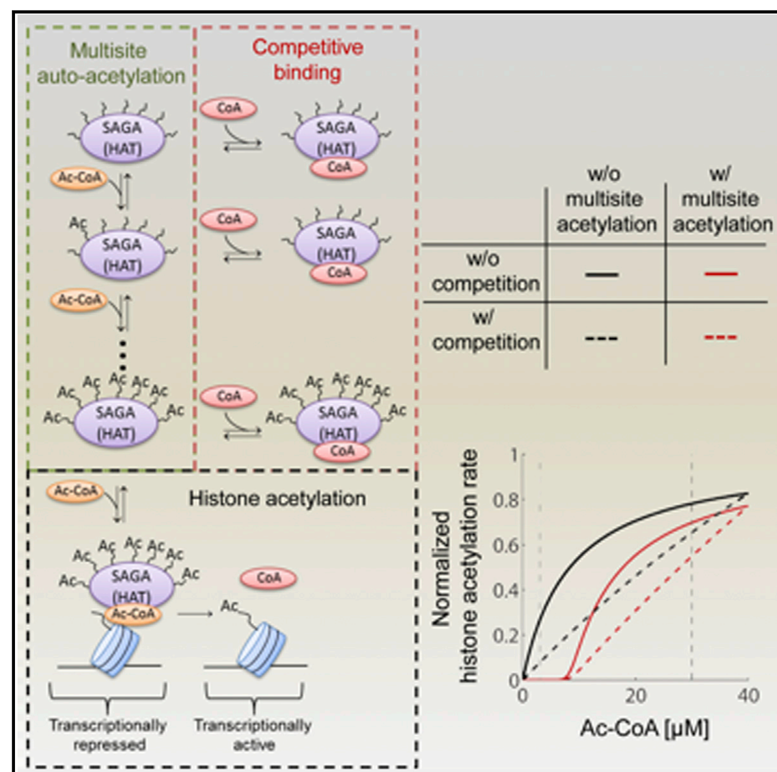
View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Cell Systems
Math | Bio

Competitive Inhibition Can Linearize Dose-Response and Generate a Linear Rectifier

Graphical Abstract



Highlights

- Competitive binding can create a linear response that does not saturate
- Competitive binding coupled with positive feedback can generate a linear rectifier
- Histone acetylation meets the conditions required to behave as a linear rectifier
- The requirements for constructing this type of linear rectifier are commonly met

Authors

Yonatan Savir, Benjamin P. Tu, Michael Springer

Correspondence

michael_springer@hms.harvard.edu

In Brief

Savir et al. show how competitive inhibition can result in a linear response that, when combined with positive feedback, can generate a linear rectifier. This work highlights how linear rectifiers can be achieved with biologically simple and potentially common motifs.

Savir et al., 2015, Cell Systems *1*, 238–245
September 23, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.cels.2015.09.001

CellPress

CellPress
Cell Systems
Math | Bio

Competitive Inhibition Can Linearize Dose-Response and Generate a Linear Rectifier

Yonatan Savir,[1] Benjamin P. Tu,[2] and Michael Springer[1,*]
[1]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA
[2]Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390, USA
*Correspondence: michael_springer@hms.harvard.edu
http://dx.doi.org/10.1016/j.cels.2015.09.001

SUMMARY

Many biological responses require a dynamic range that is larger than standard bi-molecular interactions allow, yet have the ability to remain off at low input. Here, we mathematically show that an enzyme reaction system involving a combination of competitive inhibition, conservation of the total level of substrate and inhibitor, and positive feedback can behave like a linear rectifier—that is, a network motif with an input-output relationship that is linearly sensitive to substrate above a threshold but unresponsive below the threshold. We propose that the evolutionarily conserved yeast SAGA histone acetylation complex may possess the proper physiological response characteristics and molecular interactions needed to perform as a linear rectifier, and we suggest potential experiments to test this hypothesis. One implication of this work is that linear responses and linear rectifiers might be easier to evolve or synthetically construct than is currently appreciated.

INTRODUCTION

Organisms need to respond to changes in their environment to survive. Response to these changes often involves complex signaling pathways that process information about the environment and directly or indirectly regulate protein concentration and activity. The enzyme kinetics that underlie cellular signaling are typically bimolecular, resulting in a Michaelis-Menten relationship between reactants (inputs) and products (outputs) (Figure 1A) (Fersht, 1985). This relationship has the disadvantage that it saturates at high input levels, therefore the output is most responsive when the input concentration is low (Figure 1B). In isolation, this property cannot create the rich signal processing observed in nature (e.g., oscillators or excitable systems). Therefore, signaling pathways combine bimolecular reactions together in ways that result in more complex input-output functions. For example, a switch-like response can be achieved using enzyme cooperativity (Ferrell, 1996; Qian, 2012). An ideal switch is maximally sensitive at a threshold concentration of input and is unresponsive at concentrations both above and below this threshold (Figure 1C). This response can be advantageous in situations where only one of two outcomes is desired. For example, switches are used to make cell-fate decisions (Santos and Ferrell, 2008).

While switches are useful, there are many situations where it could be desirable to respond above a minimal threshold but still be responsive to graded concentrations of input well above this threshold. A shifted Michaelis-Menten relationship (a Michaelis-Menten response translated to the right along the substrate axis; see black curve in Figure 1C) does not respond below a threshold, is maximally responsive at the threshold, but is still responsive above this threshold. This type of response can be achieved by several mechanisms, such as multisite phosphorylation (Gunawardena, 2005) or a suicide inhibitor (Fersht, 1985). For example, if an inhibitor existed that irreversibly binds the substrate and sequesters it, there could be no response until the substrate concentration was higher than the inhibitor (Elf et al., 2005; Levine et al., 2007; Mehta et al., 2008). At this point, the system would behave as if the inhibitor was not there, but the effective concentration of substrate would be the actual concentration minus the concentration of inhibitor. While responsive over a wider dynamic range than a switch-like response, this response still saturates at high input values, and thus the sensitivity to input decreases as input increases above the threshold (Figure 1D).

Signal saturation is a classical engineering problem. One solution is to insert a “pre-distorter” between the input and a system that saturates. If the input-output relationship of the pre-distorter is the inverse of the rest of the system, the resulting input-output relationship will be linear (Becskei, 2009). This type of linearization was demonstrated in a synthetic circuit by the addition of a specific type of negative feedback and negative regulation (Nevozhay et al., 2009). While linear over a broad dynamic range, this system was not intrinsically thresholded, making it sensitive to low concentrations of input and saturated at high input values.

In contrast to shifted Michaelis-Menten response, a hybrid between the threshold and linear response, termed a linear rectifier, could guarantee that the system has no response below a threshold concentration and is linearly responsive above that threshold concentration. Linear rectifiers could in principle be extremely useful in responses where low levels of input should be “ignored” but the response needs to be maximally responsive over as wide a range of inputs as possible (Figures 1C and 1D). Nutrient regulation of growth rate and gene expression is a plausible system in which a linear rectifier could be useful (see Science Application for discussion). A linear rectifier could prove useful under any circumstance when a thresholded response is necessary, but linear relationship between input and output is also required over a larger than Michaelian range.

CrossMark

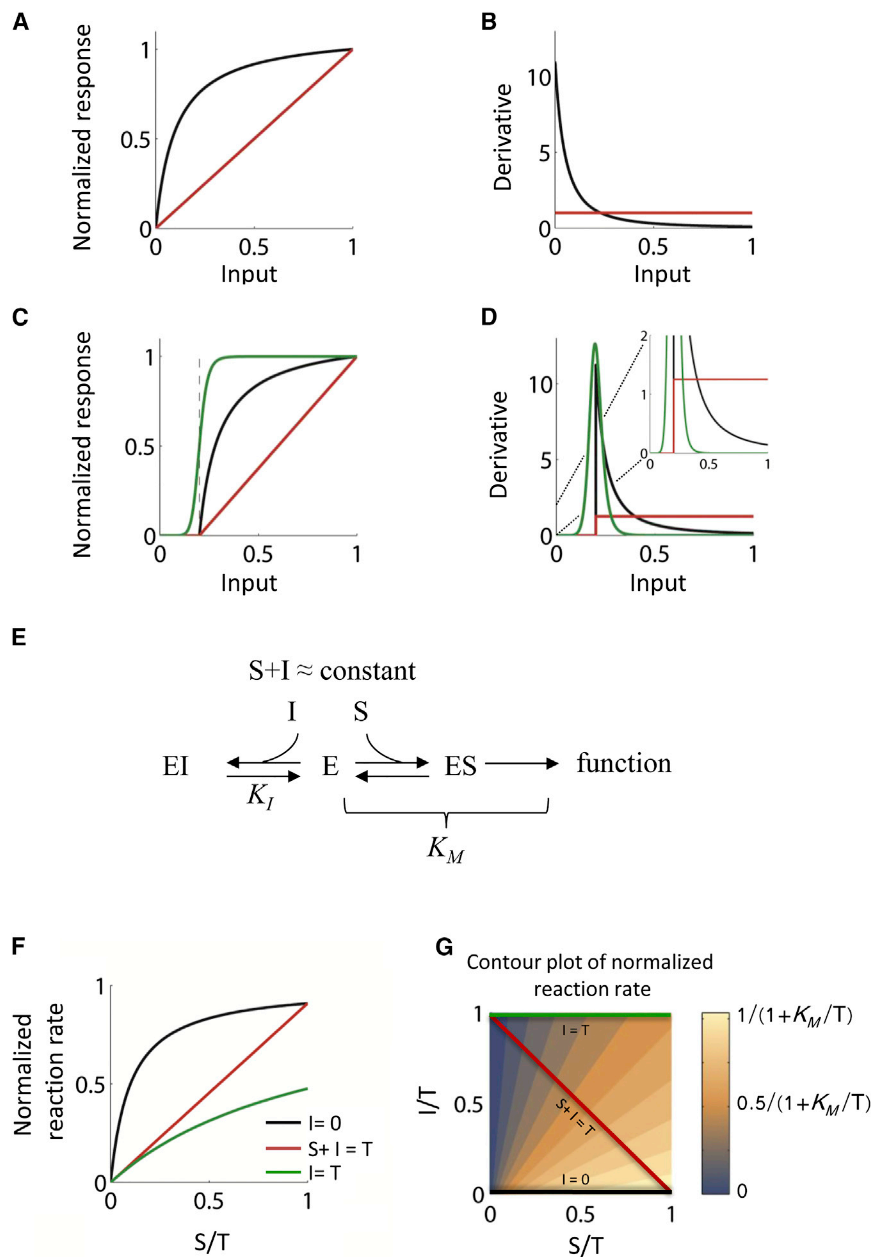


Figure 1. Competitive Inhibition Combined with Mass Conservation Leads to a Linear Relationship between the Substrate Concentration and Reaction Rate

(A–D) Typical response functions and their sensitivities. (A) Michaelian response (black) and a linear response (red) and (B) their derivatives. The derivative of the linear response highlights that it is sensitive over the entire input range while the Michaelian response saturates at high input values. Neither of these responses have a threshold. (C) Several example rectifiers. Rectifiers are unresponsive below a threshold (dashed horizontal line). Ideal Michaelian rectifier (black), ideal linear rectifier (red), and a Hill function with n = 10 (green) are given along with their derivatives (D). The Hill function and the Michaelian rectifier are akin to a step-like response; each has an "on" and "off" regime with a clear threshold and low sensitivity in each regime. However, the linear rectifier has a threshold that defines an "off" regime while being sensitive to the input across the whole "on" regime; the switch is not sensitive to changes in input concentration within the "on" regime.

(E) Kinetic schema of a general enzymatic reaction that is prone to competitive inhibition. K_M is the Michaelis-Menten constant of the substrate and K_I is the inhibitor dissociation constant.

(F) When the inhibitor levels are constant (black and green lines), the response is Michaelian. When the total substrate and inhibitor levels are constant, i.e., [S] + [I] = T, the response is linear throughout the entire range of the substrate (red line).

(G) A contour plot of the normalized reaction rate. The dependence of the reaction rate on the substrate and inhibitor levels can be represented as trajectories in the S – I plane. When inhibitor levels are constant (black and green lines) these trajectories are parallel to the substrate axis. When the total level of the substrate and inhibitor is constant (green line), the trajectory has a slope of −1; this slope results in a linearized response.

Here, we examine an enzyme reaction system that functions as a linear rectifier. It contains two features: (1) There is a strong competitive inhibition of the substrate, namely, the binding affinities of the inhibitor and substrate are similar, and (2) the total inhibitor and substrate concentration remain roughly constant. This motif had been carefully characterized in the context of ATP, ADP, and AMP binding to enzymes where the ATP binding is much stronger than that of ADP and AMP (Atkinson, 1968). In this regime, the system can behave ultrasensitively to changes in ATP levels. We extend previous work on this motif by analytically showing that it can produce a response that is linear across its full dynamic range without saturating. This motif is robust to modest perturbations in this criterion and in the need to maintain a constant concentration of inhibitor plus substrate. Additionally, we show that combining this motif with a threshold mechanism or positive feedback creates a linear rectifier.

As an example of plausibility, we explain how these features could be biologically implemented in the context of histone acetylation and propose a series of experiments that could test this hypothesis (Science Application). While we describe this motif in the context of histone acetylation, the basic design could be ubiquitous.

RESULTS

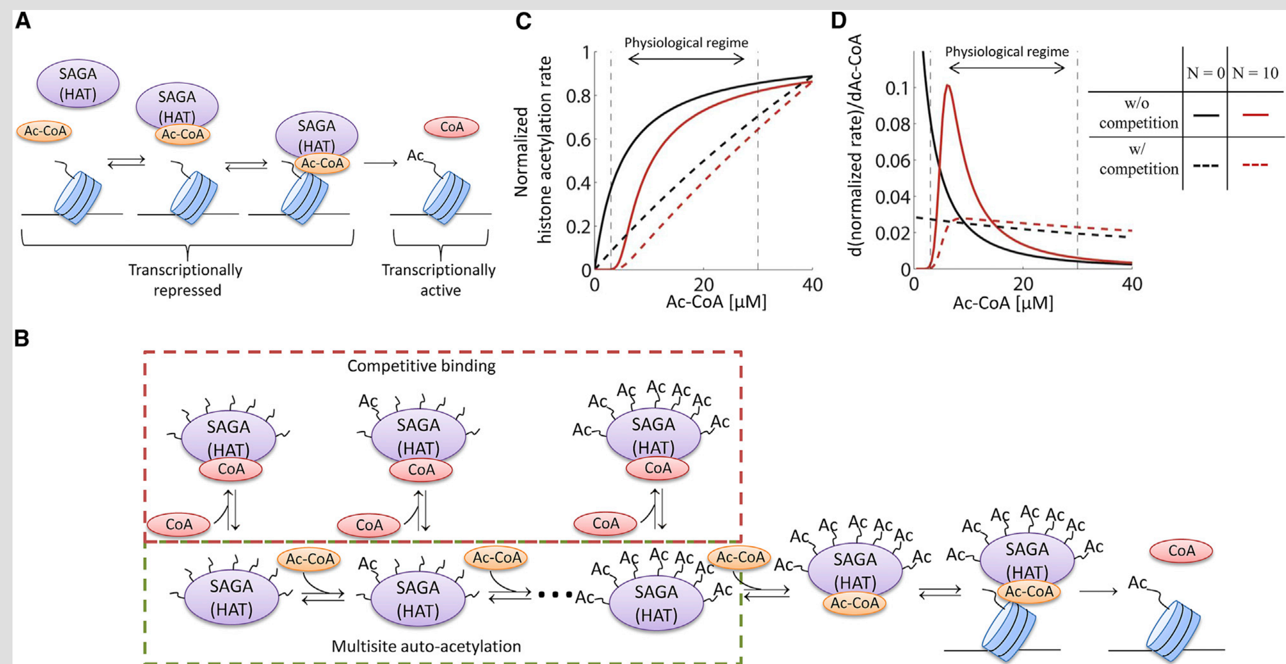
Competition between Substrate and an Inhibitor That Are Conserved Can Lead to a Linear Response

In the case of competitive binding between a substrate and an inhibitor (Figure 1E), the normalized reaction rate ($\tilde{v}$) is (Supplemental Experimental Procedures, section I) (Fersht, 1998)

Science Application. Biological Case Study: Histone Acetylation

When cells are faced with low nutrient environments, they tend to not grow; as nutrient levels are increased, the growth rate increases proportionally (Yuan et al., 2013). Several studies have suggested that gene expression for a number of genes linearly depends on growth. Expression of 60%–90% of genes is correlated with growth rate, implying that there is a global correlation between growth rate and gene expression (Keren et al., 2013). The tight coupling between growth rate and transcription is still not fully understood (Wellen and Thompson, 2012).

A potential mechanistic link could be histone acetylation (Cai et al., 2011; Guan and Xiong, 2011; Haigis and Sinclair, 2010; Morrish et al., 2010; Schwer and Verdin, 2008; Takahashi et al., 2006; Wellen et al., 2009). Acetylation of histones is a key transcriptional regulatory step that can have a large effect on gene expression (Wellen and Thompson, 2012). Ac-CoA is the source of the acetyl group used by histone acetyl transferases (HAT) and it is also a key metabolite whose level is affected by nutrients and energy availability, with levels increasing during growth (Cai et al., 2011). Ac-CoA increases have been shown to result in increased acetylation of histones on genes involved in growth, potentially providing a direct link between growth rate and transcription (Cai et al., 2011). Here, we outline how the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex from *Saccharomyces cerevisiae*, a key histone acetyl transferase (HAT) that is conserved from yeast to human (Berndsen and Denu, 2008; Koutelou et al., 2010; Tanner et al., 2000), could be a concrete example of a biological linear rectifier.



Histone Acetylation Has the Potential to Behave as a Linear Rectifier

(A) Acetylation of histones involves the serial sequential formation of a ternary complex between SAGA, Ac-CoA, and histone.

(B–D) CoA competes with Ac-CoA (top) and multisite auto-acetylation can provide switch-like character (bottom) to the formation of catalytically competent SAGA. Together, this provides a response that has both a sharp threshold and a linear regime (C and D).

It Is Plausible That Saga Responds Linearly to Changes in Ac-CoA Concentration

Acetylation of histones by SAGA (Grant et al., 1997; Kuo et al., 1996) involves the serial sequential formation of a ternary complex between SAGA, Ac-CoA, and histone (Berndsen and Denu, 2008) (Science Application and S1; Supplemental Experimental Procedures). Two products result from the acetylation reaction: acetylated histone and CoA. Competition for Gcn5 binding between Ac-CoA and CoA may lead to a linear response, given that the following two conditions are met. Condition 1: the CoA dissociation constant for Gcn5, $K_{D,C}$, (6.7 μM; Fan et al., 2015; Tanner et al., 2000), is very close to the dissociation rate for Ac-CoA, $K_{D,A}$ (5.1 μM). In vivo, CoA and Ac-CoA concentrations are similar both in *Escherichia coli* (Chohnan et al., 1998) and in mammalian cells (Lee et al., 2014). Thus, CoA is a physiologically relevant inhibitor of Ac-CoA in multiple organisms (Fan et al., 2015; Tanner et al., 2000). Condition 2: the total concentration of Ac-CoA and CoA is conserved. Two lines of evidence support the possibility that in vivo CoA and Ac-CoA levels are interdependent. First, Ac-CoA and CoA levels are mechanistically coupled as there are a number

(Continued on next page)

Science Application. ***Continued***

of cellular reactions that interconvert the two species. Second, Ac-CoA and CoA levels were measured in *E. coli*; while the ratio of Ac-CoA and CoA changed by over 10-fold across several different environmental conditions, the combined amount of these two metabolites varied by under 50% (Chohnan et al., 1998).

When Combined with Thresholding, a Linear Saga Response Could Produce a Linear Rectifier

By analogy to multisite phosphorylation, multisite acetylation can impose an activity threshold. In *Saccharomyces cerevisiae*, at least three components of the SAGA complex are acetylated by the catalytic subunit of SAGA, Gcn5p. Based on chromatin immunoprecipitation, acetylated SAGA interacts more strongly to a ribosomal gene during growth phase relative to the non-acetylated SAGA, suggesting that the auto-acetylation of SAGA might play a role in enhancing its capacity as an acetyltransferase (Cai et al., 2011). In addition, auto-acetylation has been suggested to affect histone acetyl transferase (HAT) activity in other systems (Yuan and Marmorstein, 2013). A reaction scheme that combines multisite auto-acetylation with conservation of the total levels of Ac-CoA and CoA and competitive binding (Science Application) leads to a response that has both a sharp threshold and a linear regime (Science Application), i.e., a linear rectifier (Supplemental Experimental Procedures, section V).

Future Experimental Tests

There are several experiments that could help establish SAGA as a linear rectifier. In vitro experiments with purified components can establish whether the SAGA complex is capable of a linear response. While a number of in vitro enzymatic assays have been performed with SAGA, both in the absence and presence of CoA, unusual experiments need to be performed to identify a linear motif. Specifically, assays must be conducted at multiple different CoA levels while the total amount of Ac-CoA and CoA is held constant. To know whether an intrinsic ability to respond linearly is used in vivo, a different approach must be taken because direct control over intracellular Ac-CoA and CoA is difficult if not impossible. Therefore, the intracellular concentration of Ac-CoA needs to be measured in a number of different environments and compared to the amount of histone acetylation. This will directly measure whether the histone acetylation rate is linearly proportional to Ac-CoA. Further support for the proposed mechanism would be given if the total of Ac-CoA and CoA is nearly constant across conditions. Mutants such as *acs1* that affect the level of CoA, Ac-CoA, or their ratio would help to separate whether the linear response is directly controlled by CoA/AcCoA levels or by some other nutrient-dependent signaling pathway.

Additionally, three classes of mutants should exist: (1) mutants that affect the binding of CoA and AcCoA equivalently, (2) mutants that affect CoA and AcCoA binding differentially, and (3) mutants that affect feedback. The first and third class should shift the activation threshold without compromising the linearity of response; the first class would change the slope of the linear response. These two classes might be achieved, respectively, with a natural variant in gcn5 that affects binding e.g., *gcn5 A190T* (Langer et al., 2002) and acetylation site mutants of SAGA. This change in threshold should be noticeable in experiments measuring growth rates in different, especially poor, nutrient environments. Additionally, these mutants could have significantly higher cell death at low nutrient levels that should be testable by viability assays. The second class of mutant could potentially be rationally designed with the aid of the crystal structure of Gcn5 (Trievel et al., 1999). A mutant that bound AcCoA much better than CoA might be expected to saturate growth at a lower external nutrient concentration, which should be measurable by competition with wild-type strains in high nutrient environments. These potential decoupling mutants could allow for a deeper mechanistic examination of the regulation of growth control.

$$\tilde{v} = \frac{[S]}{[S] + K_M + [I]\frac{K_M}{K_I}}, \quad \text{(Equation 1)}$$

where K_M is the Michaelian constant for the substrate and K_I is the dissociation constant for the inhibitor. Competition does not change the V_{max} of the reaction; instead the effective affinity constant for the reaction is changed. Although the presence of inhibitor affects the reaction rate, if the inhibitor levels are constant the response as a function of substrate is still Michaelian (Figure 1F). However, if the substrate and inhibitor levels are interdependent, a non-Michaelian response is possible, e.g., if the sum of the substrate and inhibitor is constant (Atkinson, 1968). If T = [S] + [I], Equation 1 can be rewritten as,

$$\tilde{v} = \frac{[S]}{[S]\left(1 - \frac{K_M}{K_I}\right) + K_M + T\frac{K_M}{K_I}}. \quad \text{(Equation 2)}$$

When the substrate and inhibitor dissociation constants are similar, $K_M \approx K_I$, the response becomes (Supplemental Experimental Procedures, section I),

$$\tilde{v} \approx \frac{[S]}{K_M + T}. \quad \text{(Equation 3)}$$

Since K_M and T are both constants, the response is linear with respect to substrate over the entire physiological range of substrate concentrations (Figure 1F).

To understand the relationship between the substrate and the reaction rate when the substrate and inhibitor levels are interdependent, it is helpful to visualize the problem as a two-dimensional surface (Figure 1G). As the substrate level changes, the trajectory on the S − I plane in Figure 2C depends on the relationship between the substrate and inhibitor.

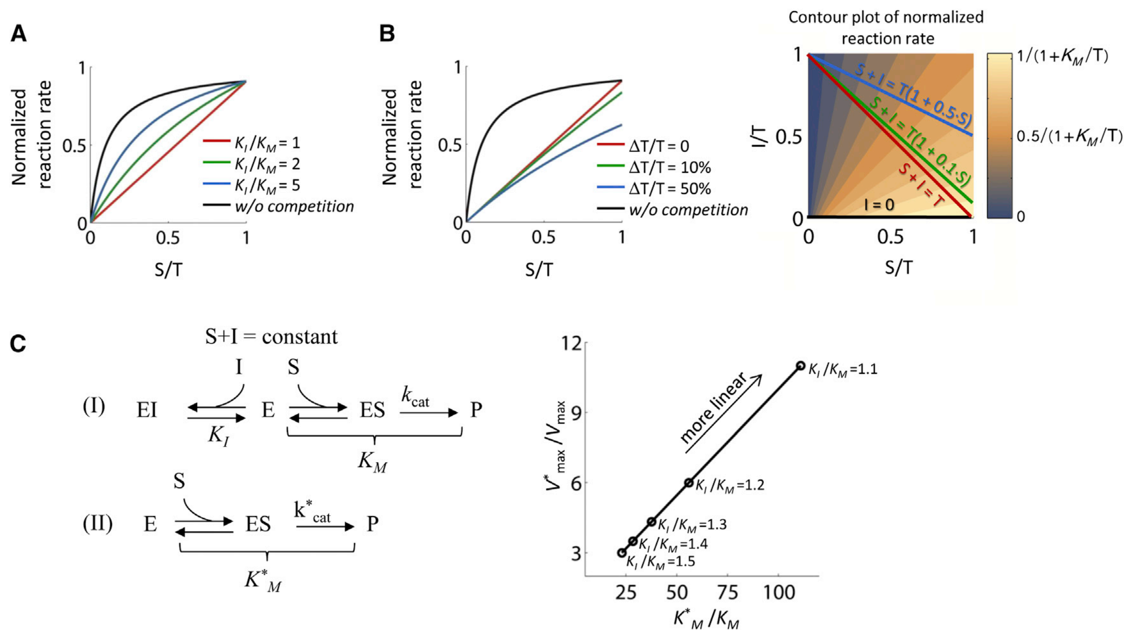


Figure 2. Deviations from Ideal Linearization

(A) The case in which the substrate affinity is higher than the inhibitor affinity, i.e., K_I/K_M is larger than one.

(B) The case in which the total concentration of the substrate and the inhibitor is not constant but rather increases with the substrate levels. As a result, trajectories in the S − I plane might have a slope other than −1.

(C) The kinetics constant that lead a linear (I) and Michaelian (II) reaction to behave equivalently in the physiological range. Where $K_I/K_M = 1.3$ and $T/K_M = 10$ (similar to the case for the Ac-CoA/CoA system (Science Application), see text for general relation). A Michaelian scheme (II) needs an affinity that is ~37 times larger and a maximal velocity that is ~4.5 times larger than the competitive inhibition scheme (I).

Horizontal lines in this plane i.e., constant inhibitor, result in a Michaelian relationship between input and reaction rate (green and black lines in Figures 1F and 1G). Diagonal lines in this plane, S + I = constant, result in a linear relationship between the substrate and the reaction rate (red line in Figures 1F and 1G).

While the system is only linear when both $K_M = K_I$ and [S] + [I] = constant, modest deviations from these equalities are still approximately linear (Figure 2A), even when there are variations in the total sum of the substrate and inhibitor (Figure 2B; Supplemental Experimental Procedures, section I). Michaelian relationships are also approximately linear when the substrate concentration is well below the K_M of the reaction. It is therefore possible to recapitulate the response of competitive inhibition with conservation of the total levels of substrate and inhibitor with a standard Michaelian reaction (Figure 2C), but this will come at the expense of making the reaction less efficient per unit enzyme (Figure 2C; Supplemental Experimental Procedures, section II). For example, to achieve the same response in a scenario where K_I/K_M = 1.3 and T/K_M = 10 (similar to the case for the Ac-CoA/CoA system, Science Application) a Michaelian system would need an ~37-fold higher K_M and require V_{max} to increase ~4.5-fold (Figure 2C). In general, the affinity of a Michaelian system required to match the linear motif is $K^*_M = K_M\left(\frac{T/K_M + K_I/K_M}{K_I/K_M - 1}\right)$ and its maximal velocity is $V^*_{max} = V_{max}\left(\frac{K_I/K_M}{K_I/K_M - 1}\right)$ where the constants without stars are from the linear motif.

Competitive Binding Combined with a Thresholding Mechanism Can Lead to a Linear Rectifier

The linear motif described above does not intrinsically have a threshold. General mechanisms, such as positive feedback (Alon, 2007) or multisite modification (Gunawardena, 2005), can create a threshold. We therefore asked whether combining the linear motif with these thresholding motifs could create a linear rectifier.

We first considered whether positive feedback can convert a linear response to a linear rectifier. One simple realization of positive feedback in a general catalysis scheme can be achieved by making the enzyme complex increase the level of enzyme (Figure 3A). In this case, the resulting reaction is a shifted Michaelian with a threshold that depends on the strength of the feedback and the enzyme affinity (Figures 3A–3C; Supplemental Experimental Procedures, section III). Combining competitive inhibition with conservation of the total levels of substrate and inhibitor shifts the threshold toward higher substrate concentrations and linearizes the response (Figures 3B and 3C; Supplemental Experimental Procedures, section III), thus resulting in a linear rectifier.

Second, we tested whether a second thresholding mechanism, multisite auto-activation, could also convert a linear response to a linear rectifier (Figure 3D). In the case of phosphorylation, experimental and theoretical studies (Ferrell, 1996; Gunawardena, 2005; Huang and Ferrell, 1996) have found that multisite phosphorylation in a signaling cascade can lead to a threshold behavior if multiple modifications are needed for the

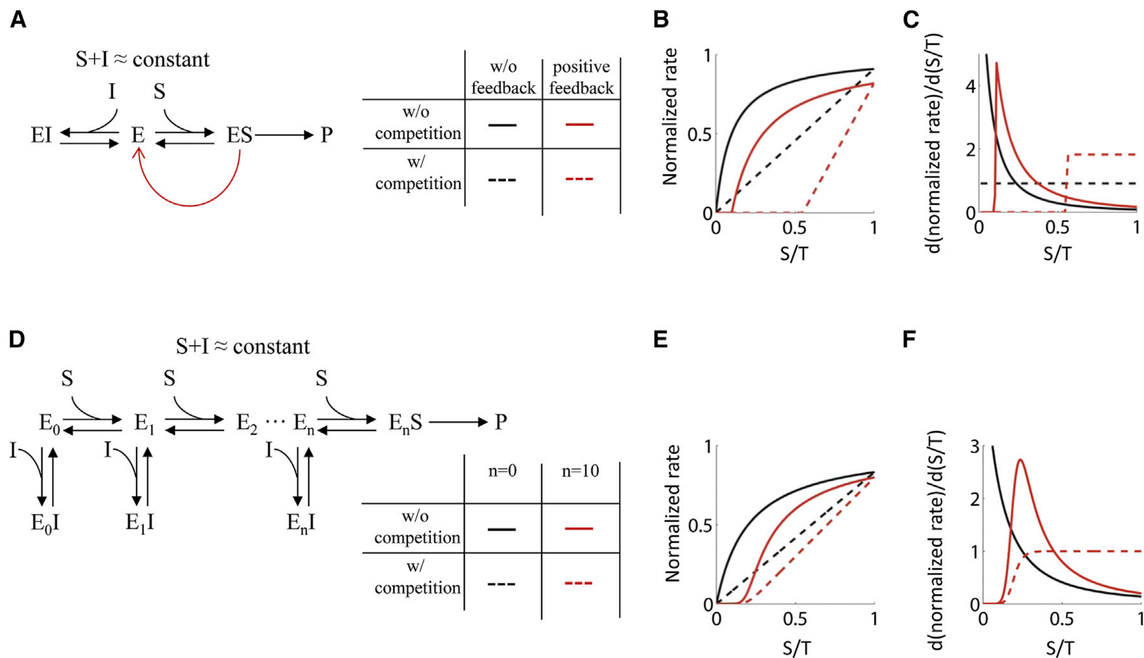


Figure 3. Conversion of a Linear Response to a Linear Rectifier

(A) A competitive inhibition linearization motif that includes positive feedback.

(B) Relationship between substrate and reaction rate with (red) and without (black) positive feedback and with (dashed) or without (solid) competitive inhibition linearization.

(C) The rate derivates show how combining competitive binding with substrate-inhibitor interplay and positive feedback leads to a response that is not sensitive below a threshold and does not saturate above it.

(D) Reaction scheme with auto-modification and competitive inhibition linearization motif.

(E and F) Reaction rate (E) and its derivative (F) with (dashed) and without (solid) competitive binding and with (red) and without (black) multi-site auto-modification.

kinase's substrate to gain its activity. This situation is analogous to multisite acetylation. In the Science Application, we discuss the example of histone acetyl transferase auto-acetylation. In its simplest scenario, the ordered distributive case, auto-modification results in a shifted Michaelian response. The sharpness of the response depends on the number of auto-activation steps (Figure 3E; Supplemental Experimental Procedures, section IV). Combining competitive inhibition with substrate-inhibitor conservation retains the threshold of the response but linearizes above the threshold (Figure 3E; Supplemental Experimental Procedures, section IV). As in the case of the positive feedback, auto-modification converts a linear response into a linear rectifier.

DISCUSSION

In this study, we found that a relatively simple biological motif is capable of creating a response that is linear throughout the entire physiological range of substrate without saturating. The motif requires three features: (1) the reaction has a competitive inhibitor, (2) the total concentration of substrate and inhibitor is conserved, and (3) the affinities of the inhibitor and substrate for the enzyme are the same. This linear response can be combined with thresholding mechanism (e.g., positive feedback or multisite modification) to create a linear rectifier—a reaction that is linearly responsive over the full dynamic range when the substrate is above a threshold. Both rectification and linearization of a response have been described in other reaction schemes. For example, "suicide inhibition" approximates a rectifier (Elf et al., 2005; Levine et al., 2007; Mehta et al., 2008) and negative feedback with sequestration can result in a linearized response (Becskei, 2009; Nevozhay et al., 2009). Here, we show that a common motif, competitive binding, can in certain conditions have the underappreciated function of linearizing the response over the full dynamic range. Additionally, we show how this motif, when combined with thresholding mechanism such as positive feedback or multisite auto-modification, can lead to linear rectifier.

We propose histone acetylation as a plausible biological system that could behave as a linear rectifier (Science Application). A number of physiological and mechanistic experiments suggest that the system might have a linear response and the mechanistic properties required of linear rectifiers. However, the experiments that would be required to convincingly show that this system behaves as a linear motif or a linear rectifier have not been performed. This is not because testing for linearization and linear rectifiers is inherently difficult, but likely because it requires different experiments than are standard (Science Application), for example, in vitro experiments where both the substrate and inhibitor are co-titrated. There are multiple labs that study Spt-Ada-Gcn5-acetyltransferase (SAGA) that are well positioned for confirming or refuting this hypothesis with respect to histone acetylation.

Many biological reactions form products that have very similar structures to one of the reactants and therefore have the potential to serve as conserved competitive inhibitors. In the case of histone acetylation, this inhibition could result from any of the components of the reaction: the co-factor (e.g., SAM/SAH), a recycled product (e.g., NAD^+/NAM), or even the substrate (e.g., a peptide/phosphopeptide). This inhibition may shape metabolic responses in a non-trivial ways (Escalante-Chong et al., 2015). While the relative ratio of substrate and product can change, the timescale of signaling is usually much quicker than changes in total metabolite concentration or protein, making mass conservation likely in many systems. In total, given the numerous ways that the necessary features for a linearizer and linear rectifier can be created, there are potentially numerous systems that may have these behaviors.

Linear responses and linear rectifiers have properties that can be useful in a number of biological processes. Linear responses allow for systems to be both proportionally responsive and have high total reaction rates without needing high enzyme levels. This could be a desirable property when the environment could vary over a wide range. For example, this type of linear response might have an advantage in the context of growth regulation: it allows a clear threshold that separates a non-growing state from a growing state while at the same time allows a sensitive growth adjustment in the growth state. Linear rectifiers also have very useful signal processing properties. The thresholding behavior naturally filters noise while the linear response maximizes information transfer; again this could be useful in a process like growth control where a cell might not want to commit to growth when nutrients are below a certain level. Additionally, linear rectifiers have been shown to be more versatile than sigmoidal functions in creating a wide range of output responses in neural networks (Nair and Hinton, 2010), suggesting that linear rectifiers might play useful roles as modules inside more complicated metazoan signaling networks. In total, our work highlights how linear rectifiers can be achieved with biologically simple and potentially common motifs.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cels.2015.09.001.

AUTHOR CONTRIBUTIONS

Y.S., B.P.T., and M.S. conceived and designed the study. Y.S. and M.S. developed the analytical and numerical framework and wrote the paper.

ACKNOWLEDGMENTS

We would like to thank S. Boswell, C.M. DeGennaro, L. Cai, L. Shi, and members of the M.S. lab for valuable discussions. R. Milo provided a critical feedback on the manuscript. This study was supported by the National Science Foundation grant 1349248 and the P50 GM107618 (M.S.), the Human Frontier Science Program (Y.S.), and award R01GM094314 from the National Institute of General Medical Sciences (B.P.T.).

Received: May 4, 2015
Revised: August 21, 2015
Accepted: September 2, 2015
Published: September 23, 2015

REFERENCES

Alon, U. (2007). Network motifs: theory and experimental approaches. Nat. Rev. Genet. *8*, 450–461.

Atkinson, D.E. (1968). The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry *7*, 4030–4034.

Becskei, A. (2009). Linearization through distortion: a new facet of negative feedback in signalling. Mol. Syst. Biol. *5*, 255.

Berndsen, C.E., and Denu, J.M. (2008). Catalysis and substrate selection by histone/protein lysine acetyltransferases. Curr. Opin. Struct. Biol. *18*, 682–689.

Cai, L., Sutter, B.M., Li, B., and Tu, B.P. (2011). Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell *42*, 426–437.

Chohnan, S., Izawa, H., Nishihara, H., and Takamura, Y. (1998). Changes in size of intracellular pools of coenzyme A and its thioesters in Escherichia coli K-12 cells to various carbon sources and stresses. Biosci. Biotechnol. Biochem. *62*, 1122–1128.

Elf, J., Paulsson, J., Berg, O., and Ehrenberg, M. (2005). Mesoscopic kinetics and its applications in protein synthesis. In Systems Biology, F.A. Alberghina and H.V. Westerhoff, eds. (Springer), pp. 95–108.

Escalante-Chong, R., Savir, Y., Carroll, S.M., Ingraham, J.B., Wang, J., Marx, C.J., and Springer, M. (2015). Galactose metabolic genes in yeast respond to a ratio of galactose and glucose. Proc. Natl. Acad. Sci. USA *112*, 1636–1641.

Fan, J., Krautkramer, K.A., Feldman, J.L., and Denu, J.M. (2015). Metabolic regulation of histone post-translational modifications. ACS Chem. Biol. *10*, 95–108.

Ferrell, J.E., Jr. (1996). Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. Trends Biochem. Sci. *21*, 460–466.

Fersht, A.R. (1985). Enzyme Structure and Mechanism, Second Edition (W.H. Freeman).

Fersht, A.R. (1998). Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding (W.H. Freeman).

Grant, P.A., Duggan, L., Côté, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., et al. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev. *11*, 1640–1650.

Guan, K.L., and Xiong, Y. (2011). Regulation of intermediary metabolism by protein acetylation. Trends Biochem. Sci. *36*, 108–116.

Gunawardena, J. (2005). Multisite protein phosphorylation makes a good threshold but can be a poor switch. Proc. Natl. Acad. Sci. USA *102*, 14617–14622.

Haigis, M.C., and Sinclair, D.A. (2010). Mammalian sirtuins: biological insights and disease relevance. Annu. Rev. Pathol. *5*, 253–295.

Huang, C.-Y., and Ferrell, J.E., Jr. (1996). Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc. Natl. Acad. Sci. USA *93*, 10078–10083.

Keren, L., Zackay, O., Lotan-Pompan, M., Barenholz, U., Dekel, E., Sasson, V., Aidelberg, G., Bren, A., Zeevi, D., Weinberger, A., et al. (2013). Promoters maintain their relative activity levels under different growth conditions. Mol. Syst. Biol. *9*, 701.

Koutelou, E., Hirsch, C.L., and Dent, S.Y. (2010). Multiple faces of the SAGA complex. Curr. Opin. Cell Biol. *22*, 374–382.

Kuo, M.H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature *383*, 269–272.

Langer, M.R., Fry, C.J., Peterson, C.L., and Denu, J.M. (2002). Modulating acetyl-CoA binding in the GCN5 family of histone acetyltransferases. J. Biol. Chem. *277*, 27337–27344.

Lee, J.V., Carrer, A., Shah, S., Snyder, N.W., Wei, S., Venneti, S., Worth, A.J., Yuan, Z.-F., Lim, H.-W., Liu, S., et al. (2014). Akt-dependent metabolic

reprogramming regulates tumor cell histone acetylation. Cell Metab. *20*, 306–319.

Levine, E., Zhang, Z., Kuhlman, T., and Hwa, T. (2007). Quantitative characteristics of gene regulation by small RNA. PLoS Biol. *5*, e229.

Mehta, P., Goyal, S., and Wingreen, N.S. (2008). A quantitative comparison of sRNA-based and protein-based gene regulation. Mol. Syst. Biol. *4*, 221.

Morrish, F., Noonan, J., Perez-Olsen, C., Gafken, P.R., Fitzgibbon, M., Kelleher, J., VanGilst, M., and Hockenbery, D. (2010). Myc-dependent mitochondrial generation of acetyl-CoA contributes to fatty acid biosynthesis and histone acetylation during cell cycle entry. J. Biol. Chem. *285*, 36267–36274.

Nair, V., and Hinton, G.E. (2010). Rectified linear units improve restricted Boltzmann machines. In Proceedings of the 27th International Conference on Machine Learning (ICML-10), pp. 807–814.

Nevozhay, D., Adams, R.M., Murphy, K.F., Josić, K., and Balázsi, G. (2009). Negative autoregulation linearizes the dose-response and suppresses the heterogeneity of gene expression. Proc. Natl. Acad. Sci. USA *106*, 5123–5128.

Qian, H. (2012). Cooperativity in cellular biochemical processes: noise-enhanced sensitivity, fluctuating enzyme, bistability with nonlinear feedback, and other mechanisms for sigmoidal responses. Annu. Rev. Biophys. *41*, 179–204.

Santos, S.D., and Ferrell, J.E. (2008). Systems biology: on the cell cycle and its switches. Nature *454*, 288–289.

Schwer, B., and Verdin, E. (2008). Conserved metabolic regulatory functions of sirtuins. Cell Metab. *7*, 104–112.

Takahashi, H., McCaffery, J.M., Irizarry, R.A., and Boeke, J.D. (2006). Nucleocytosolic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription. Mol. Cell *23*, 207–217.

Tanner, K.G., Langer, M.R., Kim, Y., and Denu, J.M. (2000). Kinetic mechanism of the histone acetyltransferase GCN5 from yeast. J. Biol. Chem. *275*, 22048–22055.

Trievel, R.C., Rojas, J.R., Sterner, D.E., Venkataramani, R.N., Wang, L., Zhou, J., Allis, C.D., Berger, S.L., and Marmorstein, R. (1999). Crystal structure and mechanism of histone acetylation of the yeast GCN5 transcriptional coactivator. Proc. Natl. Acad. Sci. USA *96*, 8931–8936.

Wellen, K.E., and Thompson, C.B. (2012). A two-way street: reciprocal regulation of metabolism and signalling. Nat. Rev. Mol. Cell Biol. *13*, 270–276.

Wellen, K.E., Hatzivassiliou, G., Sachdeva, U.M., Bui, T.V., Cross, J.R., and Thompson, C.B. (2009). ATP-citrate lyase links cellular metabolism to histone acetylation. Science *324*, 1076–1080.

Yuan, H., and Marmorstein, R. (2013). Histone acetyltransferases: rising ancient counterparts to protein kinases. Biopolymers *99*, 98–111.

Yuan, H.-X., Xiong, Y., and Guan, K.-L. (2013). Nutrient sensing, metabolism, and cell growth control. Mol. Cell *49*, 379–387.