Supplementary information S2 (box) | Feedback designs that turn a universal covalent-modification cycle into bistable switch and relaxation oscillator.

A universal motif of cellular networks is the cycle that is formed by two forms of a signalling protein, which is modified by two opposing enzymes, such as a kinase and phosphatase, or a guanine nucleotide exchange factor (GEF) and a GTPase–activating protein (GAP). Assuming that the total concentration of these two forms (M and M_p) of the protein remains constant $M_p + M = M_{tot} = const,$ the temporal dynamics of this system is described by a single differential equation,

$$\frac{dM_p}{dt} = v_{kin} - v_{phos} \qquad \text{(S1)}$$

This simple system can turn bistable in four distinct ways; either M_p activates v_{kin} or inhibits v_{phos}, or M activates v_{phos} or inhibits v_{kin}. All these feedback designs are equivalent to positive (autocatalytic) feedback in equation S1, by which M_p or M activate their own production rates. The figure, part A in BOX 2 of the main text shows hysteresis of the dependence of M_p on the kinase concentration E_{kin}, where the values of kinetic parameters (equation 1) are the following, $k_{kin}^{cat} = 1 s^{-1}$; $A = 100$; $K_a = 500\, nM$; $K_{m1} = 500\, nM$; $E_{phos} = 200$ nM, $k_{phos}^{cat} = 1 s^{-1}$; $K_{m2} = 10\, nM$.

For each of the four bistable switches, eight different forms of negative feedback give rise to 32 different feedback designs that might exhibit relaxation oscillations, in which M_p inhibits its production rate through the negative or positive influence of either form (M and M_p) on the abundance of the kinase protein or phosphatase protein, (FIG. 2 of the main text and supplementary information S2 (box)). The temporal dynamics of each of these simple positive-negative feedback systems can be described by two differential equations (although in FIG. 2 and supplementary information S3 (figure) each diagram formally corresponds to a system of three differential equations, the protein concentration that is not controlled by feedback from the M cycle can be considered as a constant parameter, leaving only two differential equations in each case). When the abundance of the kinase protein is subject to feedback regulation by M or M_p, these equations are (see equation 2 in BOX 2 of the main text and supplementary information S2 (box)),

$$\frac{dM_p}{dt} = v_{kin} - v_{phos},$$

$$\frac{dE_{kin}}{dt} = v_{kin}^{synth} - v_{kin}^{\deg} \qquad \text{(S2)}.$$

If the abundance of the phosphatase protein is controlled by feedback (see equation 3 in BOX 2 of the main text and supplementary information S3 (figure)), this phosphorylation cycle is described by the two following equations,

$$\frac{dM_p}{dt} = v_{kin} - v_{phos},$$

$$\frac{dE_{phos}}{dt} = v_{phos}^{synth} - v_{phos}^{\deg} \qquad \text{(S3)}.$$

Supplementary information S2 (box) gives the rate expressions and kinetic parameters that generate relaxation oscillations in the universal signalling cycle for a subset of sixteen positive-negative feedback designs shown in FIG. 1 of the main text (using these rate expressions, equations S2 and S3 can be readily analysed and the emergence of oscillations can be shown). The remaining sixteen relaxation oscillation designs shown in supplementary information S3 (figure) might require some degree of cooperativity of feedback regulations.

This table provides the rate expressions and kinetic parameters that correspond to the 16 feedback designs (from **A** to **H***) shown in FIG. 2 of the main text.

A-D. Bistability in the M phosphorylation/dephosphorylation cycle arises from positive feedback (activation of the kinase rate by its product M_p) that operates on the time scale of seconds to minutes (equation 1). $M_{tot} = M_p + M = 300$. The rate expressions and kinetic parameters of this bistable M cycle are given below.

Reaction	**Rate expression**	**Kinetic constants**
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$	$k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500 nM;$ $K_{m1} = 500 nM;\ E_{kin} = 80 nM$
Phosphatase $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$	$k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10 nM;$ $E_{phos} = 200 nM$

A. Relaxation oscillations arise from additional negative feedback brought about by inhibition of the kinase protein synthesis rate by the phosphorylated form M_p (see equations 1 and 2 of the main text), which can occur at a slower time scale (tens of minutes for immediate early genes and hours for gene expression circuits).

Reaction	Rate expression	Kinetic constants
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$	$k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\, nM;$ $K_{m1} = 500\, nM;$
Phosphatase $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$	$k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\, nM;$ $E_{phos} = 200 nM$
Kinase synthesis $\rightarrow E_{kin}$	$v_{kin}^{synth} = V_{kin}^0 \frac{(1 + M_p / K_I)}{(1 + I \cdot M_p / K_I)}$	$V_{kin}^0 = 150 nM \cdot hr^{-1}; K_I = 100\, nM;$ $I = 7.5$
Kinase degradation $E_{kin} \rightarrow$	$v_{kin}^{deg} = k_{kin}^{deg} E_{kin}$	$k_{kin}^{deg} = 1 hr^{-1}$

B. Relaxation oscillations arise from extra negative feedback brought about by activation of the kinase protein degradation rate by M_p.

Reaction	Rate expression	Kinetic constants
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$	$k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\, nM;$ $K_{m1} = 500\, nM;$
Phosphatase $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$	$k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\, nM;$ $E_{phos} = 200 nM$
Kinase synthesis $\rightarrow E_{kin}$	$v_{kin}^{synth} = V_{kin}^0$	$V_{kin}^0 = 150 nM \cdot hr^{-1}$
Kinase degradation $E_{kin} \rightarrow$	$v_{kin}^{deg} = k_{kin}^{deg} \frac{(1 + A_{dk} M_p / K_d)}{(1 + M_p / K_d)} E_{kin}$	$k_{kin}^{deg} = 1 hr^{-1}; K_d = 100\, nM; A_{dk} = 7.5$

C. Relaxation oscillations arise from extra negative feedback brought about by activation of the phosphatase protein synthesis rate by the active form M_p (equations 1 and 3 of the main text).

Reaction	Rate expression	Kinetic constants
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$	$k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\, nM;$ $K_{m1} = 500\, nM; E_{kin} = 150\, nM$
Phosphatase $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$	$k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\, nM$
Phosphatase synthesis $\rightarrow E_{phos}$	$v_{phos}^{synth} = V_{phos}^0 \frac{(1 + A_p M_p / K_d)}{(1 + M_p / K_d)}$	$V_{phos}^0 = 200 nM \cdot hr^{-1}; K_d = 100\, nM;$ $A_p = 7.5$
Phosphatase degradation $E_{phos} \rightarrow$	$v_{phos}^{deg} = k_{phos}^{deg} E_{phos}$	$k_{phos}^{deg} = 1 hr^{-1}$

D. Relaxation oscillations arise from extra negative feedback brought about by inhibition of the phosphatase protein degradation rate by the active form M_p.

Reaction	Rate expression	Kinetic constants
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$	$k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\,nM;$ $K_{m1} = 500\,nM; E_{kin} = 150\,nM$
Phosphatase $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$	$k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\,nM$
Phosphatase synthesis $\rightarrow E_{phos}$	$v_{phos}^{synth} = V_{phos}^{0}$	$V_{phos}^{0} = 200\,nM \cdot hr^{-1};$
Phosphatase degradation $E_{phos} \rightarrow$	$v_{phos}^{\deg} = k_{phos}^{\deg} \frac{(1 + M_p / K_I)}{(1 + I \cdot M_p / K_I)} E_{phos}$	$k_{phos}^{\deg} = 1 hr^{-1}; K_I = 100\,nM;$ $I = 7.5$

E-H. Bistability in the M phosphorylation/dephosphorylation cycle is brought about by positive feedback in the M_p production cycle. This feedback is the result of inhibition of the phosphatase rate by its substrate M_p and usually operates on the time scale of seconds to minutes. The rate expressions and kinetic parameters of the M cycle with this feedback are given below. $M_{tot} = M_p + M = 300$.

Reaction	Rate expression	Kinetic constants
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)}$	$k_{kin}^{cat} = 1 s^{-1}; K_{m1} = 500\,nM$ $E_{kin} = 80\,nM$
Phosphatase: $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)} \cdot \frac{(1 + M_p / K_s)}{(1 + I_s \cdot M_p / K_s)}$	$k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\,nM;$ $I_s = 100; K_s = 500\,nM;$ $E_{phos} = 200\,nM$

For **E-H** designs, relaxation oscillations arise from extra negative feedback, brought about by the influence of the phosphorylated form M_p on the rates of protein synthesis or degradation of the kinase or phosphatases. If the effect is on the kinase synthesis or degradation rates, E_{phos} = 200 nM, and if it is on the phosphatases synthesis or degradation rates, E_{kin} = 150 nM. These reactions might occur at a slower time scale (tens of minutes for immediate early genes and hours for gene expression circuits. For feedback designs **E, F, G,** and **H**, the rate expressions and kinetic parameters of the kinase/phosphatase synthesis and degradation rates are the same as for feedback designs **A, B, C,** and **D**, respectively, which are given above.

Designs **A*-H*** are mirror images of designs **A-H** and have exactly the same equation structure and parameter values. The differences are that the variables M_p and E_{kin} and their respective parameters are replaced by M and E_{phos} and their respective parameters, and vice versa. For instance, the rate expressions and parameters for design **A*** are the following:

Reaction	**Rate expression**	**Kinetic constants**
Kinase: $M \rightarrow M_p$	$v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m2} + M)}$	$k_{kin}^{cat} = 1\,s^{-1}; K_{m2} = 10\,nM$; $E_{kin} = 200\,nM$
Phosphatase $M_p \rightarrow M$	$v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m1} + M_p)} \cdot \frac{(1 + AM/K_a)}{(1 + M/K_a)}$	$k_{phos}^{cat} = 1\,s^{-1}; K_{m1} = 500\,nM$; $A = 100$; $K_a = 500\,nM$
Phosphatase synthesis $\rightarrow E_{phos}$	$v_{phos}^{synth} = V_{phos}^{0} \frac{(1 + M/K_I)}{(1 + I \cdot M/K_I)}$	$V_{phos}^{0} = 150\,nM \cdot hr^{-1}; K_I = 100\,nM$; $I = 7.5$
Phosphatase degradation $E_{phos} \rightarrow$	$v_{phos}^{deg} = k_{phos}^{deg} E_{phos}$	$k_{phos}^{deg} = 1\,hr^{-1}$