Supplement S5: The Shared Inhibitor Motif

In the main text we showed that competitive inhibition of Casp3 and Casp9 by XIAP can bring about positive feedback and bistability in the intrinsic apoptosis pathway (see Fig. 2B, grey line; Fig. 4F). Similar conclusions regarding positive feedback and bistability also hold in general if an inhibitory protein competitively inhibits two consecutive intermediates in signal transduction cascades. This 'shared inhibitor motif' is schematically depicted in Fig. S5. A stimulus, S, activates the upstream intermediate, U, which then in turn catalyzes the activation of the downstream intermediate, D. Both active intermediates, U* and D*, are subject to negative regulation by the shared inhibitor, I. As indicated in Fig. S5, the shared inhibitor can either be a stoichiometric inhibitor of the intermediates (black arrows in Fig. S5) or alternatively catalyzes their deactivation, e.g., dephosphorylation (black *and* grey arrows in Fig. S5).

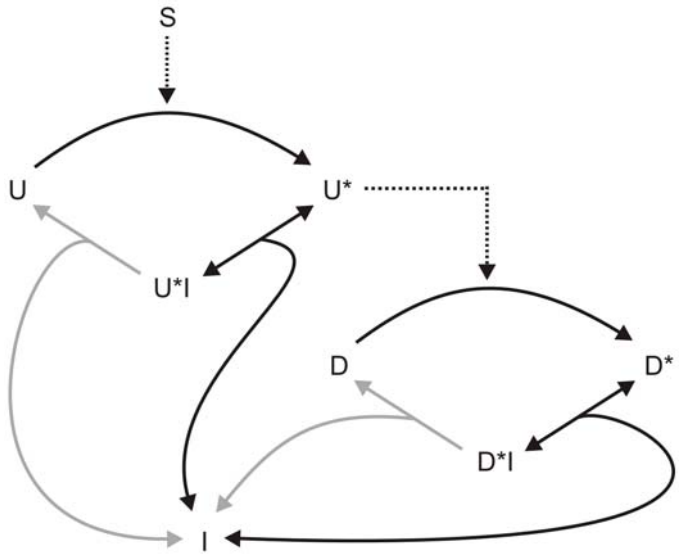

Figure S5: The Shared Inhibitor Motif

In general, bistability can arise if the shared inhibitor binds the intermediates competitively at least to some extent. Furthermore, bistability requires that *only* the active downstream intermediate, D*, but not its precursor, D, binds to the inhibitor, I. In addition to these 'structural' requirements, the downstream intermediate, D, needs to be significantly more abundant than the inhibitor, I, which in turn must exceed the upstream intermediate, U (see main text). Finally, the inhibitor, I, mediates particularly strong positive feedback if the downstream intermediate exceeds the dissociation constant (or the Michaelis-Menten constant) of the D*I-complex.
It should be noted that shared inhibitors, which function enzymatically (black *and* grey arrows in Fig. S5), can be efficiently sequestered by the downstream intermediate, D*, and thereby mediate positive feedback and bistability even if the D*I is only transiently formed and then broken down by catalysis (as long as the Michaelis-Menten constant is low enough).

Table S3 gives an overview on signal transduction pathways, where the 'shared inhibitor motif' has been reported to occur. Inhibitory proteins were subclassified into three groups, according to their biochemical mechanism of action: (i) stoichiometric inhibitors (Inh.), which reversibly sequester proteins away from their cellular targets; (ii) GTPase-activating proteins (GAP), that stimulate the intrinsic GTPase activity of small G proteins, and thereby catalyze their deactivation; (iii) Phosphatases (PP), which antagonize protein-phosphorylation cascades, are the most prominent group in Table S3.

Upstream (U)	Downstream (D)	Shared Inhibitor(s) (I)	Type	Refs.
Caspase-9	Caspase-3	X-IAP, c-IAP1/2, Survivin	Inhib.	6
Caspase-9	Caspase-7	X-IAP, c-IAP1/2, Survivin	Inhib.	6
FGFR	Mek	Sef	Inhib.	7
Grb-2	Raf	Sprouty	Inhib.	7
CyclinD / CDK4/6	Cyclin E / CDK2	p21-CIP1, p27-KIP1, p57-KIP2	Inhib.	8
Daxx (-> Ask1)	Cytochrome C	Hsp27	Inhib.	9,10
Cdc42	Rac	10 different GAPs reported	GAP	11,12
Rac	Rho	4 different GAPs reported	GAP	11,12
Cdc42	Rho	5 different GAPs reported	GAP	11,12
insulin receptor	IRS-1	LAR	PP	13
EGF receptor	HGF receptor	LAR	PP	14,15
CaMK II	AMPAR	PP1	PP	16
Aurora-B kinase	Histone 3	PP1	PP	17
Aurora-B kinase	Ndc10	PP1	PP	17
NMDA receptor	CaMK II	PP1	PP	17
p38	Caspase-3	PP2A	PP	18
Mek	Erk	PP2A	PP	19
PKA	CREB	PP2A	PP	20-22
Mek/Erk	CREB	PP2A	PP	20-22
Akt	CREB	PP2A	PP	20-22
CaMK IV	CREB	PP2A	PP	20-22
SEK1	JNK	PP2A	PP	10,22
Akt	p70S6K	PP2A	PP	21-23
PKA	Mek/Erk	PP2A	PP	21,22,24
PKCα	Mek/Erk	PP2A	PP	27,28,25
MKK6	p38	PP2C-α	PP	26
insulin receptor	IRS-1	PTP-1B	PP	13
Epo receptor	Jak2	PTP-1B	PP	27,28
Epo receptor	STAT5a/b	PTP-1B	PP	27,29
EGFR	IGF-1 receptor	PTP-1B	PP	14,30
IGF-1 receptor	EGF receptor	PTP-1B	PP	14,31
Jak2	EGF receptor	PTP-1B	PP	14,28,32
insulin receptor	Jak2	PTP-1B	PP	14,28,33,34
insulin receptor	STAT5a/b	PTP-1B	PP	14,29,33,34
PDGF receptor	Jak2	PTP-1B	PP	14,28,35
PDGF receptor	STAT5a/b	PTP-1B	PP	29,32,35
CAKβ	p130(CAS)	PTP-PEST	PP	36
CAKβ	Paxillin	PTP-PEST	PP	36
EGFreceptor	p52Shc	TCPTP	PP	37

Table S3: The ‘Shared Inhibitor motif’ is a recurrent motif in cellular signal transduction

Available experimental data suggest that bistability due to sequestration of a shared phosphatase can occur *in vivo*. Most phosphatases exhibit a single active site, i.e., they bind their substrates in a competitive manner. Additionally, they usually recognize only phosphorylated, but not non-phosphorylated, substrates, so that the structural requirements mentioned above are fulfilled. Quantitative measurements of protein abundance in the MAPK cascade revealed that the downstream intermediates in this system are (much) more abundant when compared to their upstream activators [1,2]. Finally, many phosphatases exhibit Michaelis-Menten constants in the sub-micromolar range [3,4], which suggests that strong feedback can be established (see above).

The feedback mechanism proposed in this paper may, for example, contribute to bistability in the JNK cascade [5], since PP2A was shown to dephosphorylate both JNK and its upstream activator SEK1 (see Table S3).

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