

Signal Event	Stimulus	Condition	Signal Duration	Reference
Erk phosphorylation	Growth Factors	Cell culture	10' – 24h	(Yamamoto <i>et al</i> , 2006) (Wu <i>et al</i> , 1994)
Erk phosphorylation	Antigenic peptides	Thymocyte selection (<i>in vivo</i>)	> 70 h	(McNeil <i>et al</i> , 2005)
Erk phosphorylation	Developmental Signals	Mouse development (<i>in vivo</i>)	up to 72 h	(Corson <i>et al</i> , 2003)
PI3K activity	insulin	Cell culture	10' - 8h	(Balbis <i>et al</i> , 2000; Rose <i>et al</i> , 1998)
Akt phosphorylation	NGF	Cell culture	10' - 15h	(Balbis <i>et al</i> , 2000; Chang <i>et al</i> , 2003)
cAMP increase or PKA activation	Isoprotenerol	Cell culture	5' - 2 h	(Gharami and Das, 2004; Violin <i>et al</i> , 2008)
Smad2 phosphorylation	TGFbeta	Cell culture	2 h – 24 h	(Nicolas and Hill, 2003; Zhu <i>et al</i> , 2005)
Nuclear Smad2-Smad4 complex formation	TGFbeta	Cell culture	2 h – 6 h	(Nicolas <i>et al</i> , 2003)
Nuclear Translocation of Smad2	TGFbeta	Cell culture	2 h – 6 h	(Nicolas <i>et al</i> , 2003)
Smad2 DNA binding	TGFbeta	Cell culture	2 h – 6 h	(Nicolas <i>et al</i> , 2003)
Smad2 phosphorylation	TGFbeta	Liver regeneration (<i>in vivo</i>)	> 120h	(Macias-Silva <i>et al</i> , 2002)
STAT activation	Cytokines	Cell culture	Minutes to several hours	(Brysha <i>et al</i> , 2001; Buettner <i>et al</i> , 2002)
STAT3 DNA binding	Cytokines	Liver regeneration (<i>in vivo</i>)	8 h	(Cressman <i>et al</i> , 1996)
NF-kB DNA binding	Cytokines	Cell culture	1 h – 6 h	(Hoffmann <i>et al</i> , 2002; Werner <i>et al</i> , 2005)
NF-kB DNA binding	Cytokines	Liver regeneration (<i>in vivo</i>)	~ 10 h	(Kovalovich <i>et al</i> , 2000)

Table S1: Signal durations of intracellular signaling pathways. The time a given signal remains significantly elevated over the basal level was estimated from literature studies (see references)

Signaling event	Stimulus	Cell Type	Inhibitor	Duration (no inhibitor)	Reference
NF- κ B binding to DNA	IL1beta	epithelial	ActD	~320'	(Hershko <i>et al</i> , 2004)
NF- κ B binding to DNA	IL1beta	epithelial	CHX	~320'	(Hershko <i>et al</i> , 2004)
Smad1/5 phosphorylation	TGFbeta	BAECs	CHX	~120'	(Valdimarsdottir <i>et al</i> , 2006)
STAT3 binding to DNA	IL-10	Macrophages	ActD	~60'	(Niemand <i>et al</i> , 2003)
STAT5 phosphorylation	G-CSF	32D	ActD	~20'	(Zhuang <i>et al</i> , 2005)
STAT5 phosphorylation	G-CSF	32D	CHX	~20'	(Zhuang <i>et al</i> , 2005)
STAT5 binding to DNA	G-CSF	32D	ActD	~20'	(Zhuang <i>et al</i> , 2005)
STAT5 binding to DNA	G-CSF	32D	CHX	~20'	(Zhuang <i>et al</i> , 2005)
Erk phosphorylation	EGF	Swiss 3T3	CHX	~45'	(Alessi <i>et al</i> , 1995)
Erk phosphorylation	serum	fibroblasts	CHX	~120'	(Sun <i>et al</i> , 1993)
Erk phosphorylation	EGF	HeLa	CHX	~30'	(Amit <i>et al</i> , 2007)
Erk phosphorylation	HRG	MCF7	CHX	~120'	(Nagashima <i>et al</i> , 2007)
Nuclear Erk phosphorylation	serum	CCL39	ActD	?	(Volmat <i>et al</i> , 2001)
Nuclear Erk phosphorylation	serum	CCL39	CHX	?	(Volmat <i>et al</i> , 2001)
Erk kinase activity	Angiotensin II	VSMC	ActD	~60'	(Duff <i>et al</i> , 1995)
JNK phosphorylation	EGF	HeLa	CHX	~30'	(Amit <i>et al</i> , 2007)
JNK phosphorylation	H ₂ O ₂	293T	ActD	~240'	(Teng <i>et al</i> , 2007)
JNK phosphorylation	H ₂ O ₂	293T	CHX	~240'	(Teng <i>et al</i> , 2007)
JNK kinase activity	TNFalpha	Mesangial	ActD	~20'	(Guo <i>et al</i> , 1998)
JNK kinase activity	TNFalpha	Mesangial	CHX	~20'	(Guo <i>et al</i> , 1998)
p38 phosphorylation	EGF	HeLa	CHX	~30'	(Amit <i>et al</i> , 2007)

Table S2: Transcription/translation inhibitors enhance the signal duration (and eventually the amplitude) of intracellular signaling pathways. Listed are literature studies where time courses of signaling protein activity was measured in the presence and in the absence of a transcription inhibitor (actinomycin D = ActD) and/or a translation inhibitor (cycloheximide = CHX). The column 'signal duration (no inhibitor)' indicates signal duration in cells not treated with inhibitor and thus gives a hint about the time scale of transcriptional feedback regulation.

Signal Event	Cellular Response	Stimulus (cell type)	Method	Threshold time	Reference
Mek activity	S-Phase Entry	FGF (NIH3T3)	Inhibitor addition	14 h ^a	(Yamamoto <i>et al</i> , 2006)
Mek activity	S-Phase Entry	PDGF (NIH3T3)	Inhibitor addition	> 8 h ^d	(Jones and Kazlauskas, 2001)
Mek activity	S-Phase Entry	PDGF (IIC9)	Inhibitor addition	> 4 h ^a	(Weber <i>et al</i> , 1997)
PI3K activity	S-Phase Entry	insulin (Rat1)	Inhibitor addition or antibody injection	13 h ^a	(Rose <i>et al</i> , 1998)
Mek activity	Cell Motility	HGF (MDCK)	Inhibitor addition	> 30' ^b	(Tanimura <i>et al</i> , 2002)
Mek activity	Cell Motility	EGF (SCC-11F)	Inhibitor addition	4 h ^b	(McCawley <i>et al</i> , 1999)
Mek activity	Differentiation	TPA (K562)	Inhibitor addition	18 h ^b	(Racke <i>et al</i> , 1997)
Mek activity	Differentiation	M-CSF (myeloid)	Inhibitor addition	> 24 h ^b	(Gobert Gosse <i>et al</i> , 2005)
Mek activity	Thymocyte positive selection	Antigenic peptides (<i>in vivo</i>)	Inhibitor addition	> 24 h ^b	(McNeil <i>et al</i> , 2005)
TGFbeta receptor activity	Growth arrest	TGFbeta (HaCaT)	Inhibitor addition	12-14 h ^a	(Nicolas <i>et al</i> , 2003)
Calcineurin activity	Thymocyte lineage commitment	PMA + Ionomycin (thymocytes)	Inhibitor addition	~8 h ^a	(Adachi <i>et al</i> , 2000)
PKA activity	Astroglial Differentiation	Isoproterenol (astrocytes)	Inhibitor addition	< 2 h ^e	(Gharami <i>et al</i> , 2004)
Mek activity	Late-phase Gene Expression	HGF (MDCK)	Inhibitor addition	> 30' ^b	(Tanimura <i>et al</i> , 2002)
Mek activity	MMP-9 expression	EGF (SCC-11F)	Inhibitor addition	4 h ^b	(McCawley <i>et al</i> , 1999)
Erk phosphorylation	Late-phase Gene Expression	LPA (Rat-1)	Stimulus-strength specific signal duration	~1 h ^{b,c}	(Cook <i>et al</i> , 1999)

Table S3: Commitment Times in Intracellular Signaling. Listed are literature studies where activity of signaling intermediates was blocked at different stimulation times (stimulus addition at $t = 0$ h) by incubating cells with rapidly-acting small-molecule inhibitors. The threshold times indicate the signal duration required to irreversibly commit the cell population to the given phenotypic response (stimulation occurred at $t = 0$ h). One study (Cook *et al*, 1999) employed a different approach: low and high doses of LPA induce transient and sustained Erk phosphorylation, respectively, and some Erk-dependent downstream genes (e.g., Fra1) were shown to be selectively expressed upon sustained Erk activation. Superscript legend: (a) half of the cells perform cellular response if signal is terminated at the threshold time; (b) no response observed if signal is terminated at the threshold time; (c) Erk dependency of late-phase gene expression verified by Erk inhibitor; (d) half-maximal thymidine incorporation is observed if signal is terminated at the threshold time; (e) complete commitment occurred before the indicated threshold time

Stimulus	Cellular Response	Cell type	Method	Threshold time	Reference
EGF	S-Phase Entry	MDCK	Medium exchange	~6 h ^a	(Pennock and Wang, 2003)
EGF	S-Phase Entry	BT20	Medium exchange	~6 h ^a	(Pennock <i>et al</i> , 2003)
Serum	S-Phase Entry	MDCK	Medium exchange	~6 h ^a	(Pennock <i>et al</i> , 2003)
Serum	S-Phase Entry	BT20	Medium exchange	~6 h ^a	(Pennock <i>et al</i> , 2003)
Thrombin	S-Phase Entry	CCL34	Rapid Ligand Removal by Competitor	8 h ^b	(Van Obberghen-Schilling <i>et al</i> , 1982)
Forskolin	S-Phase Entry	Thyocytes	Medium exchange	20 h ^b	(Roger <i>et al</i> , 1987)
PDGF	S-Phase Entry	NIH3T3	Medium exchange (acid wash)	9 h ^c	(Jones <i>et al</i> , 2001)
Ionomycin + PMA	Thymocyte lineage commitment	Thymocytes	Medium exchange	~8 h ^a	(Adachi <i>et al</i> , 2000)
FGF deprivation	Differentiation	Myoblasts	Medium exchange	~2.5 h ^a	(Clegg <i>et al</i> , 1987)
NGF deprivation	Apoptosis	Sympathetic Neurons	Medium exchange	~24 h ^a	(Deshmukh <i>et al</i> , 2000)
IL-3 deprivation	Apoptosis	myeloid	Medium exchange	~24 h ^a	(Ekert <i>et al</i> , 2004)
High Glucose	Late-phase Gene Expression	Min6	Medium exchange	2-3 h ^b	(Glaser and Schlegel, 2006)
PDGF	c-myc expression	NIH3T3	Medium exchange (acid wash)	30' ^b	(Jones <i>et al</i> , 2001)
TNF-alpha	RANTES expression	Fibroblasts	Medium exchange	~2 h ^b	(Hoffmann <i>et al</i> , 2002)

Table S4: Commitment Times for Extracellular Stimulation. Listed are literature studies where extracellular stimuli were removed (or re-added) at different stimulation times, e.g., by medium exchange (see Methods). The threshold times indicate the signal duration required to irreversibly commit the cell population to the given phenotypic response (stimulation occurred at $t = 0$ h). Superscript legend: (a) half of the cells perform cellular response if signal is terminated at the threshold time; (b) no response observed if signal is terminated at the threshold time; (c) half-maximal thymidine incorporation is observed if signal is terminated at the threshold time.