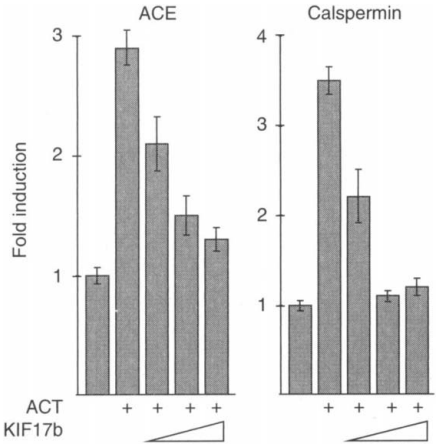

Fig. 4. KIF17b represses ACT-dependent transcriptional activation. COS cells transfected with 0.5 μg of ACE-CAT or calspermin-CAT (*2*), 2 μg of CREM and 3 μg ACT with increasing amounts of pSG5-Kif17b. Activation is reported as fold induction of CREM-induced promoter. Transfection efficiency was monitored by β-galactosidase assays using the CMVβ-gal plasmid. Data are means ±SD of results from three independent transfection experiments (*25*).

by KIF17b was observed (Fig. 4).[6] This effect is specific, as confirmed by the use of an unrelated kinesin and another transcriptional activator (VP16). It was also observed on a Gal4-based heterologous promoter (fig. S3). Sequestering ACT-KIF17b into the nucleus by LMB treatment leads to recovery of ACT-dependent transcriptional activation (fig. S3).

The findings presented here expand the view of the role that kinesins exert in cellular physiology. We have linked one kinesin directly to a transcriptional coactivator, while examples of indirect regulations were previously reported (*19*, *20*). The transduction events that govern KIF17b to directly modulate ACT-mediated transcription have yet to be explored, but another LIM-only protein with transcriptional coactivator properties, FHL2, is able to transmit Rho signals from the cell membrane to the nucleus (*21*). In addition, a mitotic kinesin-like protein was recently found to be intimately connected to Rho-dependent signaling and to be required for microtubule bundling (*22*). Growing evidence points to a functional connection between microtubule cytoskeleton integrity and transcriptional activation, as shown in the cases of factor MIZ-1 activated in response to sterols (*23*), and *Costal2*, an inhibitor of cubitus interruptus in *Drosophila* (*24*).

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Sir2-Dependent Activation of Acetyl-CoA Synthetase by Deacetylation of Active Lysine

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Acetyl-coenzyme A (CoA) synthetase (Acs) is an enzyme central to metabolism in prokaryotes and eukaryotes. Acs synthesizes acetyl CoA from acetate, adenosine triphosphate, and CoA through an acetyl–adenosine monophosphate (AMP) intermediate. Immunoblotting and mass spectrometry analysis showed that *Salmonella enterica* Acs enzyme activity is posttranslationally regulated by acetylation of lysine-609. Acetylation blocks synthesis of the adenylate intermediate but does not affect the thioester-forming activity of the enzyme. Activation of the acetylated enzyme requires the nicotinamide adenine dinucleotide–dependent protein deacetylase activity of the CobB Sir2 protein from *S. enterica*. We propose that acetylation modulates the activity of all the AMP-forming family of enzymes, including nonribosomal peptide synthetases, luciferase, and aryl- and acyl-CoA synthetases. These findings extend our knowledge of the roles of Sir2 proteins in gene silencing, chromosome stability, and cell aging and imply that lysine acetylation is a common regulatory mechanism in eukaryotes and prokaryotes.

Members of the Sir2 family of proteins (sirtuins) are nicotinamide adenine dinucleotide (NAD^+)–dependent deacetylase enzymes involved in chromosome stability, gene silencing, and cell aging in eukaryotes and archaea (*1–3*). All previously known substrates of sirtuins are components of chromatin and/or affect gene transcription. Strains of the enterobacterium *S. enterica* lacking sirtuin (encoded by the *cobB* gene) cannot grow on propionate and in low acetate concentration as carbon and energy sources because the acyl-CoA synthetases responsible for converting free acids into acyl-CoA derivatives are inactive (*4*, *5*). Our work shows that acetyl-CoA synthetase activity (Fig. 1A) (encoded by the *acs* gene) is regulated by posttranslational acetylation. We also show that activation of acetylated Acs requires CobB deacetylase activity.

Acs enzyme synthesized by a $cobB^-$ strain of *S. enterica* was inactive in crude cell-free extract (*5*). To address the possibility that Acs activity was posttranslationally regulated, we overexpressed the *acs* gene of *S. enterica* in $cobB^+$ and $cobB^-$ strains and purified the protein as described in (*6*). Acs

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protein produced by the *cobB*$^-$ strain was less active than protein isolated from the *cobB*$^+$ strain by a factor of about 100 (Table 1). Incubation of inactive Acs enzyme with purified CobB and NAD$^+$ resulted in a 480-fold increase in Acs activity; the reaction was NAD$^+$ dependent (Table 1, fig. S1). The increase in Acs activity measured in the presence of CobB and the absence of NAD$^+$ was also observed when bovine serum albumin substituted for CobB; thus, this effect appears to be due to nonspecific stabilization of Acs.

We hypothesized that the CobB-dependent activation of Acs was due to removal of acetyl groups from the inactive form of the enzyme. We took two approaches to determine the chemical nature of the modification and the site of the modification in Acs.

We performed immunoblot analysis to determine whether lysyl residues were acetylated in inactive Acs enzyme. Polyclonal antibody to acetyllysine reacted strongly with Acs enzyme isolated from a *cobB*$^-$ strain (Fig. 1B); in contrast, the same antibody lacked detectable reactivity with Acs isolated from a *cobB*$^+$ strain (Fig. 1B). The decreased reactivity of Acs with the antibody depended on the presence of NAD$^+$ and CobB in a time-course experiment, establishing a correlation between the lack of Acs activity and acetylation.

We mapped the acetylation site on inactive Acs enzyme by comparing peptide masses generated from tryptic digests of Acs protein isolated from *cobB*$^+$ and *cobB*$^-$ cells (fig. S2). The proteolytic mass fingerprints represented >74% of the amino acid sequence from Acs. The mass fingerprints for both active, deacetylated Acs and inactive, acetylated Acs (Acs^{Ac}) were virtually identical in peptide masses and their relative intensities, except for a 733.4 ion (fig. S3). This singly charged ion was qualitatively more abundant in the Acs^{Ac} preparation than in the Acs preparation, and it corresponds to the predicted mass of the SGK^{Ac}IMR^{612} peptide (*7*) with acetylation of residue Lys^{609}. We confirmed acetylation of Lys^{609} by fragmenting the 367.2 ion (the doubly charged species of the 733.4 ion) in Acs or Acs^{Ac} tryptic digests (fig. S4). A search of the nonredundant protein database yielded a single hit with the sequence SGK^{Ac}IMR^{612} from *S. enterica* Acs with a 0.01 atomic mass unit error on the peptide mass and an error of <21 parts per million on the peptide fragment masses (Table 2, fig. S5). Fragmentation occurs primarily at the peptide bond and yields a b series of ions with the charge on the NH_2-terminal amino acid and a y series of ions with the charge on the COOH-terminal amino acid. All but two of the major fragmentation ions present in the fragmentation mass spectra of the 367.2 ion were readily assigned to the SGK^{Ac}IMR^{612} sequence. Most notably, both the y_1 to y_5 and the b_2 to b_4 fragment ions were present. These ions span the acetylated Lys^{609} residue, unambiguously identifying the site of acetylation. These results show that Acs protein overproduced by the *cobB*$^-$ strain contains acetyllysine at residue Lys^{609}.

To investigate whether acetylation affected both reactions catalyzed by Acs, we took advantage of the knowledge that Acs can synthesize propionyl-CoA from propionate (*8*). Propionyl–adenosine monophosphate (AMP) (*9*, *10*) was provided as substrate in a reaction mixture containing CoA and Acs or Acs^{Ac}. Acs^{Ac} enzyme was as efficient as (specific activity = 107 μmol of product per minute per milligram of protein) if not more efficient than the Acs enzyme (specific activity = 73 μmol of product per minute per milligram of protein) in generating propionyl-CoA from propionyl-AMP and CoA. We detected no product in the absence of CoA. These results show that the thioester-forming activity of Acs^{Ac} remains unaffected by acetylation and indicate that acetylated residues in Acs^{Ac} do not affect the thioester-forming activity of the enzyme. Acetylation of the active site Lys^{609} of Acs has the same effect on Acs activity that substitutions of Lys^{592} have on propionyl-CoA synthetase activity (*10*). Lys^{609} of Acs is an invariant

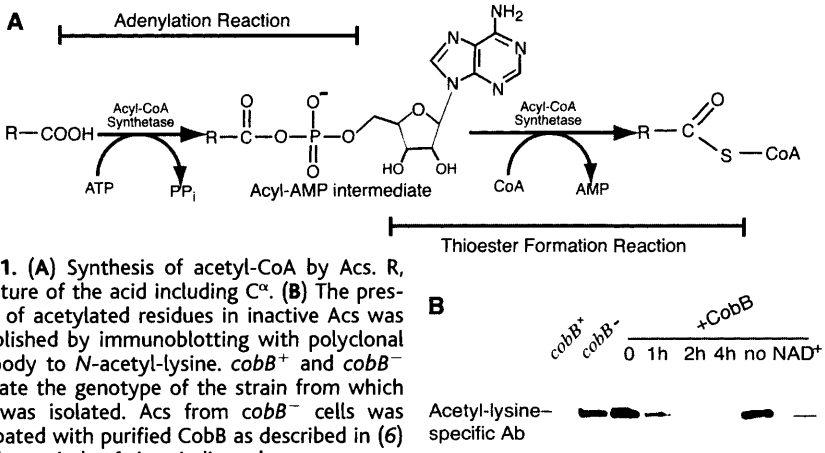


Fig. 1. (**A**) Synthesis of acetyl-CoA by Acs. R, structure of the acid including C$^\alpha$. (**B**) The presence of acetylated residues in inactive Acs was established by immunoblotting with polyclonal antibody to *N*-acetyl-lysine. *cobB*$^+$ and *cobB*$^-$ indicate the genotype of the strain from which Acs was isolated. Acs from *cobB*$^-$ cells was incubated with purified CobB as described in (*6*) for the periods of time indicated.

Table 1. NAD$^+$-, CobB-dependent activation of acetyl-CoA synthetase.

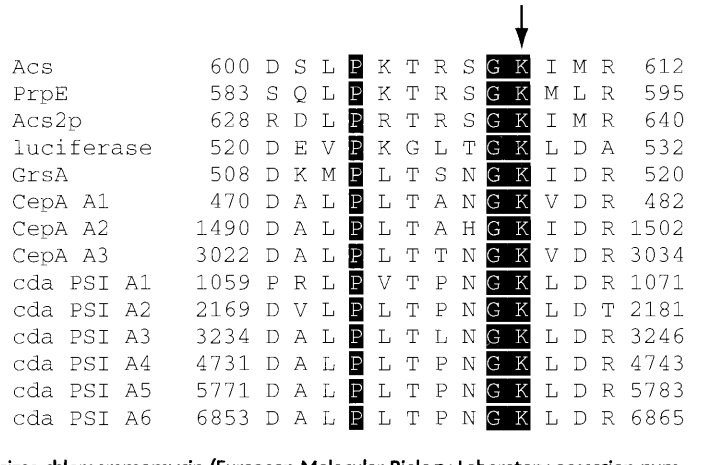
Overexpression host genotype*			
cobB$^+$		*cobB*$^-$	
Addition†	Acs specific activity‡	Addition†	Acs specific activity‡
None	119 ± 5	None	Not detectable
BSA§	351 ± 14	BSA	9 ± 0.1
CobB	806 ± 9	CobB	22 ± 4
CobB, BSA	810 ± 4	CobB BSA	19 ± 4
CobB, NAD†	809 ± 8	CobB, NAD†	480 ± 4
BSA, CobB, NAD†	902 ± 10	BSA, CobB, NAD†	483 ± 19

*Relevant genotype of the strain from which the Acs protein was purified. †All reaction mixtures contained coenzyme A, Mg(II)/ATP, and Acs proteins. ‡ Specific activity is defined as nanomoles of acetyl CoA formed per minute per milligram. Results are the average of three independent determinations. §BSA, bovine serum albumin.

Table 2. Tandem mass spectrometry analysis of the acetylated peptide of Acs.

b series *m/z**	Observed *m/z*	Sequence	y series *m/z*†	Observed *m/z*	Sequence
			y_6:733.403		SGK^{Ac}IMR
b_1: 88.040		S	y_5:646.371	646.368	GK^{Ac}IMR
b_2: 145.061	145.060	SG	y_4:589.350	589.349	K^{Ac}IMR
b_3: 315.167	315.166	SGK^{Ac}	y_3:419.244	419.242	IMR
b_4: 428.251	428.244	SGK^{Ac}I	y_2:306.160	306.157	MR
b_5: 559.291		SGK^{Ac}IM	y_1:175.119	175.118	R
b_6: 715.393		SGK^{Ac}IMR			

*Predicted fragment ion masses of SGK^{Ac}IMR with the charge on the NH_2-terminal amino acid; K^{Ac}, acetyllysine. †Predicted fragment ion masses of SGK^{Ac}IMR with the charge on the COOH-terminal amino acid.



Acs	600	D	S	L	P	K	T	R	S	G	K	I	M	R	612
PrpE	583	S	Q	L	P	K	T	R	S	G	K	M	L	R	595
Acs2p	628	R	D	L	P	R	T	R	S	G	K	I	M	R	640
luciferase	520	D	E	V	P	K	G	L	T	G	K	L	D	A	532
GrsA	508	D	K	M	P	L	T	S	N	G	K	I	D	R	520
CepA A1	470	D	A	L	P	L	T	A	N	G	K	V	D	R	482
CepA A2	1490	D	A	L	P	L	T	A	H	G	K	I	D	R	1502
CepA A3	3022	D	A	L	P	L	T	T	N	G	K	V	D	R	3034
cda PSI A1	1059	P	R	L	P	V	T	P	N	G	K	L	D	R	1071
cda PSI A2	2169	D	V	L	P	L	T	P	N	G	K	L	D	T	2181
cda PSI A3	3234	D	A	L	P	L	T	L	N	G	K	L	D	R	3246
cda PSI A4	4731	D	A	L	P	L	T	P	N	G	K	L	D	R	4743
cda PSI A5	5771	D	A	L	P	L	T	P	N	G	K	L	D	R	5783
cda PSI A6	6853	D	A	L	P	L	T	P	N	G	K	L	D	R	6865

Fig. 2. Conserved motif containing the acetylation site lysine residue among representative members of the AMP-forming family of proteins. Acs, acetyl-CoA synthetase (gi: 16767525, *S. enterica*); PrpE, propionyl-CoA synthetase (gi: 14917034, *S. enterica*); Acs2p, acetyl-CoA synthetase (gi: 6323182, *Saccharomyces cerevisiae*); GrsA, gramicidin S synthetase I (gi: 3334467, *Brevibacillus brevis*); CepA, one of three subunits that synthesizes chloroeremomycin (European Molecular Biology Laboratory accession numbers X98690 and S46968; gi: 7522085, *Amycolotopsis orientalis*); cda PSI, calcium-dependent antibiotic peptide synthetase I (open reading frame SCO3230, *Streptomyces coelicolor*). We used the motif PX_4GK to identify putative substrates of sirtuins. gi, GenInfo Identifier.

residue of a conserved motif in the family of AMP-forming enzymes (Fig. 2).[5] Lys^{592} of propionyl-CoA synthetase, Lys^{529} of luciferase, and Lys^{517} of gramicidin synthetase 1 (equivalent to Lys^{609} of Acs) are essential for synthesis of the corresponding AMP reaction intermediate but not for the thioester-forming activity of these enzymes (*10*–*13*). We propose that acetylation modulates the activity of all the AMP-forming family of enzymes. How these enzymes are acetylated remains an open question.

We provide evidence for a broadened role of sirtuins in cell physiology that includes intermediary metabolism. Our results suggest a mechanism for linking the physiological state of the eukaryotic cell with the acetylation state of histones, a key factor in chromatin silencing and chromosome stability. Several studies implicate sirtuins in life-span control in yeast and metazoans (*14*, *15*). Similarly, manipulation of NAD^+ biosynthetic mechanisms has been shown to affect life-span (*16*). A recent study documenting the effect of caloric restriction on yeast mother cell longevity suggested that the increased longevity was causally associated with increased respiration; this life-span extension was sirtuin dependent (*17*). As the Acs enzyme produces acetyl-CoA, a key metabolite of the Krebs cycle, Acs may represent a target for life-span extension.

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7. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Supporting Online Material

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A Distinct Signaling Pathway Used by the IgG-Containing B Cell Antigen Receptor

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The immunoglobulin G (IgG)–containing B lymphocyte antigen receptor (IgG-BCR) transmits a signal distinct from that of IgM-BCR or IgD-BCR, although all three use the same signal-transducing component, Igα/Igβ. Here we demonstrate that the inhibitory coreceptor CD22 down-modulates signaling through IgM-BCR and IgD-BCR, but not that through IgG-BCR, because of the IgG cytoplasmic tail, which prevents CD22 phosphorylation. These results suggest that the cytoplasmic tail of IgG specifically enhances IgG-BCR signaling by preventing CD22-mediated signal inhibition. Enhanced signaling through IgG-BCR may be involved in efficient IgG production, which is crucial for immunity to pathogens.

B cells express the membrane-bound form of immunoglobulin (mIg) on the surface as a component of the B cell antigen receptor (BCR) (*1*, *2*), and distinct isotypes of mIg are expressed by B cells, depending on their developmental stage. Naïve B cells in the peripheral lymphoid organs express both mIgM and mIgD, whereas memory B cells in spleen and lymph nodes express mostly mIgG. B cells expressing mIgG show an enhanced response to antigen stimulation compared with those expressing mIgM and/or mIgD (*3*), suggesting that IgG-BCR transmits a signal distinct from IgM-BCR or IgD-BCR. However, all mIg isotypes associate with the common BCR signaling component Igα/Igβ, indicating that all BCRs activate the same signaling pathways (*1*, *2*).

IgM-BCR signaling is negatively regulated by inhibitory coreceptors such as CD22 and CD72 (*4*–*15*), and these coreceptors are suggested to set a signaling threshold for ligation of IgM-BCR. However, little is known about whether these core-

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