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KIN-29 SIK regulates chemoreceptor gene expression via an MEF2 transcription factor and a class II HDAC

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The expression of individual chemoreceptor (CR) genes in *Caenorhabditis elegans* is regulated by multiple environmental and developmental cues, possibly enabling *C. elegans* to modulate its sensory responses. We had previously shown that KIN-29, a member of the salt-inducible kinase family, acts in a subset of chemosensory neurons to regulate the expression of CR genes, body size and entry into the alternate dauer developmental stage. Here, we show that KIN-29 regulates these processes by phosphorylating the HDA-4 class II histone deacetylase (HDAC) and inhibiting the gene repression functions of HDA-4 and an MEF-2 MADS domain transcription factor. MEF-2 binds directly to the CR gene regulatory sequences, and is required only to repress but not activate CR gene expression. A calcineurin phosphatase antagonizes the KIN-29/MEF-2-regulated pathway to modulate levels of CR gene expression. Our results identify KIN-29 as a new regulator of MEF2/HDAC functions in the nervous system, reveal cell-specific mechanisms of action of this pathway *in vivo* and demonstrate remarkable complexity in the regulation of CR gene expression in *C. elegans*.

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Introduction

Animals modify their behavior and development in response to changing external and internal conditions. These adaptive changes are mediated by changes in the functions of individual organs and cells, and ultimately, by changes in the expression of individual genes. Gene regulatory regions must, therefore, integrate information from multiple signaling pathways so as to fine-tune the spatiotemporal levels of gene expression (Wyrick and Young, 2002; Cases and de Lorenzo, 2005).

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The *Caenorhabditis elegans* sensory nervous system provides an excellent system in which to explore the mechanisms by which developmental and environmental signals converge to regulate gene expression. Unlike vertebrate and *Drosophila* olfactory neurons, each chemosensory neuron in *C. elegans* expresses multiple G protein-coupled chemoreceptor (CR) genes, each of which may respond to distinct subsets of chemicals (Lanjuin and Sengupta, 2004). We and others have shown that regulation of CR gene expression is complex, such that in addition to developmental pathways, distinct subsets of CR genes are regulated by levels of a constitutively produced pheromone, food and neuronal activity (Troemel *et al*, 1999; Peckol *et al*, 2001; Lanjuin and Sengupta, 2002; Nolan *et al*, 2002; Tobin *et al*, 2002). How information from these pathways is integrated to modulate levels of CR gene expression is not known.

A family of proteins implicated in the regulation of gene expression in response to external signals is the AMPK/SNF1 family of Ser/Thr kinases. AMPK/SNF1 kinases play major roles in regulating cellular and whole-body energy homeostasis via activation and repression of key metabolic genes in response to cellular and pathological stress (Kahn *et al*, 2005; Kim and Lee, 2005). The salt-inducible kinases (SIKs) are members of this kinase family and, as implied by their name, were originally identified as kinases upregulated in adrenocortical cells in rats fed on high-salt diets (Wang *et al*, 1999). SIKs are also upregulated in response to ACTH signaling, and depolarization and kainate-induced seizures in adrenocortical tumor cells and in the nervous system, respectively (Feldman *et al*, 2000; Okamoto *et al*, 2004). Recently, SIK1 has also been shown to be upregulated in mouse primary hepatocytes upon fasting, and SIK and AMPK play critical roles in the regulation of hepatic gluconeogenic gene expression in response to fasting and feeding (Koo *et al*, 2005). SIK activity is further promoted by phosphorylation by upstream kinases, such as LKB1 (Lizcano *et al*, 2004). However, although SIKs are expressed in multiple cell types including neurons, their roles and targets in most tissue types have not been identified.

The MEF2 class of MADS domain transcription factors also plays an essential role in the regulation of gene expression in response to environmental signals in multiple cell types. In muscles, MEF2 regulates the expression of fiber type-specific genes in response to altered patterns of electrical stimulation (Wu *et al*, 2000; Liu *et al*, 2005), whereas MEF2 is critical for activity-dependent survival of cultured post-mitotic neurons (Mao *et al*, 1999; Li *et al*, 2001; Gaudilliere *et al*, 2002). Recently, MEF2 has also been shown to regulate synapse number in response to activity in cultured hippocampal and cerebellar granule neurons (Flavell *et al*, 2006; Shalizi *et al*, 2006). The gene regulatory functions of MEF2 are extensively modulated via interaction with different proteins in different cell types and via cell-specific post-translational mechanisms

(McKinsey *et al*, 2002; Heidenreich and Linseman, 2004; Flavell *et al*, 2006; Gregoire *et al*, 2006; Riquelme *et al*, 2006; Shalizi *et al*, 2006). In particular, MEF2 interacts directly with class II histone deacetylases (HDACs) to repress gene expression (Lu *et al*, 2000b). Although multiple kinases have been implicated in the regulation of MEF2/HDAC functions, the role of AMPK family members in MEF2/HDAC-mediated gene expression has been suggested only in skeletal muscle in response to exercise via as yet uncharacterized mechanisms (Al-Khalili *et al*, 2004; Holmes *et al*, 2005; McGee and Hargreaves, 2006).

We previously showed that the *C. elegans* mutant for the SIK homolog *kin-29* exhibits phenotypes consistent with defects in the acquisition and transduction of sensory information (Lanjuin and Sengupta, 2002). In *kin-29* mutants, the expression of a subset of CR genes is altered, and animals exhibit reduced body size and deregulated entry into the alternate dauer developmental stage (Lanjuin and Sengupta, 2002; Maduzia *et al*, 2005). All examined *kin-29* phenotypes could be rescued by expression of *kin-29* in chemosensory neurons alone, leading us to suggest that misregulation of CR gene expression in *kin-29* mutants was causal to the observed physiological abnormalities. Here, we show that KIN-29 acts by antagonizing the gene repressive functions of the *C. elegans* MEF2 ortholog MEF-2 and the class II HDAC protein HDA-4 in chemosensory neurons. We show that phosphorylation of HDA-4 by KIN-29 is essential for alleviation of repression, and that constitutive Ca^{2+} signaling partly bypasses the requirement for KIN-29 in this pathway. We demonstrate that MEF-2 interacts directly with a *cis*-regulatory element in a KIN-29-regulated CR gene promoter, but is not required to promote CR gene expression. Finally, we show that the calcineurin Ser/Thr phosphatase antagonizes the KIN-29 pathway. Taken together, our results identify an SIK family member as a new regulator of MEF2/HDAC functions in the nervous system, and provide insights into the complexity of signaling pathways that regulate CR gene expression in *C. elegans*.

Results

Mutations in mef-2 and hda-4 suppress the kin-29 phenotype of downregulated CR gene expression

To identify the mechanisms by which KIN-29 regulates CR gene expression, we carried out a suppressor screen. Expression of the *str-1* and *sra-6* CR genes is reduced, although not abolished, in *kin-29* mutants in the AWB and ASH chemosensory neurons, respectively (Lanjuin and Sengupta, 2002). *kin-29(oy39)* missense mutants carrying stably integrated copies of a *str-1*::*gfp* fusion gene were mutagenized and mutants isolated in which expression of *str-1*::*gfp* in the AWB neurons was restored to wild-type levels (Figure 1A–E). Subsequent mapping and complementation experiments indicated that a subset of the identified alleles defined two complementation groups representing the *mef-2* transcription factor and the *hda-4* class II histone deactelyase (HDAC) genes (Dichoso *et al*, 2000; Choi *et al*, 2002) (Figure 1F). *oy63* and *oy65* are predicted to result in missense mutations in highly conserved amino acids in the conserved DNA-binding MADS box domain of MEF-2 (Figure 1F). The *mef-2(gv1)* allele (Dichoso *et al*, 2000) removes most of the MADS and MEF domains that are required for MEF-2 function, and is likely a null allele (Figure 1F). *oy59* results in a premature stop codon in the first exon of *hda-4*, whereas the spontaneously arising *oy57* suppressor allele is a point mutation in the splice acceptor site of the sixth exon, which is predicted to result in a truncated HDA-4 protein lacking the HDAC domain (Figure 1F). The *hda-4(ok518)* deletion allele was obtained from the *C. elegans* Gene Knockout Consortium. Sequences encoding part of the HDAC domain are deleted in *hda-4(ok518)* (Figure 1F).

We further characterized the CR gene expression phenotypes of *mef-2* and *hda-4* mutants. Mutations in *hda-4* and *mef-2* suppressed the downregulation of *str-1*::*gfp* expression in both the *kin-29(oy39)* missense and the *kin-29(oy38)* null background at all developmental stages, although no gross effects were observed on *str-1*::*gfp* expression in a wild-type background (Table I). However, *hda-4(ok518)* failed to suppress the *str-1*::*gfp* expression phenotype in *kin-29* mutant animals. It is likely that *hda-4(ok518)* is a hypomorph, as the *str-1*::*gfp* expression phenotype of *kin-29(oy38)* mutants was fully suppressed in *hda-4(oy57)/hda-4(ok518)* transheterozygotes (Table I). Mutations in *mef-2* and *hda-4* also fully suppressed the downregulation of expression of *sra-6*::*gfp* in the ASH neurons of *kin-29* mutants (Table I), indicating that MEF-2 and HDA-4 regulate the expression of multiple CR genes in different sensory neuron subtypes. Suppression of the *str-1*::*gfp* expression phenotype was abolished upon introduction of *hda-4* or *mef-2* genomic sequences into *kin-29 hda-4* or *mef-2*; *kin-29* double mutants, respectively (Table I). The observed effects on transgene expression were confirmed by semiquantitative RT–PCR, which showed that levels of endogenous *str-1* message were comparable among *mef-2*; *kin-29* and *kin-29 hda-4* double mutants and wild-type animals, but significantly reduced, although not abolished, in *kin-29* mutants or in animals mutant for the LIM homeobox gene *lim-4* required for specification of AWB fate (Sagasti *et al*, 1999) (Figure 1E).

To determine whether loss of HDA-4 function at any developmental stage is sufficient to upregulate *str-1*::*gfp* expression in *kin-29* mutants, we fed late larval stage *kin-29* mutant animals with bacteria expressing *hda-4* dsRNA. Downregulation of *hda-4* by RNAi at late larval stages led to partial suppression of the *str-1*::*gfp* expression phenotype in adults (Table I), indicating that HDA-4 is required through adulthood to modulate CR gene expression.

Additional phenotypes of kin-29 mutants are also suppressed by mutations in mef-2 and hda-4

In addition to altered CR gene expression, *kin-29* mutants exhibit a small body size and hypersensitivity to the constitutively produced dauer pheromone, such that they inappropriately enter the dauer stage at low pheromone concentrations (Lanjuin and Sengupta, 2002; Maduzia *et al*, 2005). However, *kin-29 hda-4* and *mef-2*; *kin-29* double mutants exhibited body lengths that were not significantly different from those of wild-type animals (Figure 2A), indicating that mutations in *hda-4* and *mef-2* suppress the decreased body length phenotype of *kin-29* mutants. Interestingly, similar to *kin-29* mutants, *mef-2* single mutants were significantly smaller in body length than wild-type animals (Dichoso *et al*, 2000), whereas *hda-4* single mutants were slightly thinner than wild-type animals, but exhibited

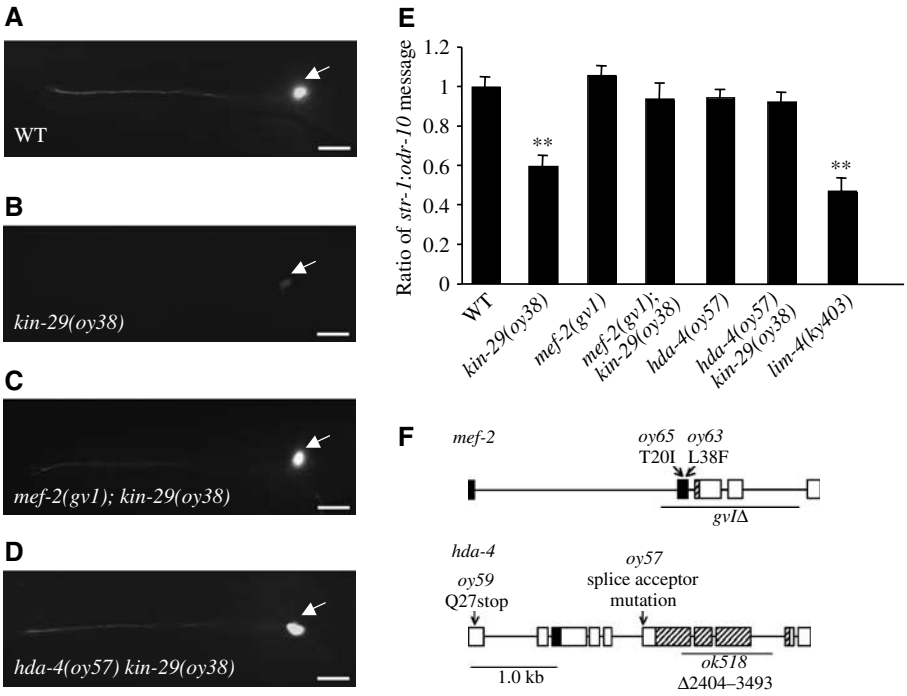

Figure 1 Mutations in the *mef-2* MEF-2 transcription factor and *hda-4* histone deacetylase genes suppress the downregulated *str-1* CR gene expression phenotype of *kin-29* mutants. (**A–D**) Expression of *str-1*::*gfp* in an AWB neuron in animals of the indicated genotypes. Arrow points to the AWB cell body. Images were acquired at the same exposure time. Lateral view: anterior is on the left. Scale, 15 μm. (**E**) Reduced levels of endogenous *str-1* message in *kin-29* mutants are restored to wild-type levels in *mef-2*; *kin-29* and *kin-29 hda-4* double mutant animals. The ratio of endogenous *str-1* message to endogenous *odr-10* message in animals of the indicated genotypes is shown. The mean of the ratios from 8 to 12 independent RT–PCR experiments performed on multiple days is shown. Error bars denote the s.e.m. Double asterisks mark levels that are different from wild type at $P<0.001$ using a two-sample *t*-test. (**F**) Genomic structures of *mef-2* and *hda-4*. The molecular identities of mutations in *mef-2* (top) and *hda-4* (bottom) are shown. The extent of the deletions in *mef-2(gv1)* and *hda-4(ok518)* are indicated by underlines. Filled and hatched boxes indicate the MADS and MEF2 domains, respectively, in MEF-2, and the MEF2-binding and HDAC domains, respectively, in HDA-4. The mutation in *oy57* results in an *hda-4* cDNA that uses an alternate splice acceptor site in intron 6, resulting in a truncated HDA-4 protein.

normal body length (Figure 2A). Mutations in *hda-4* and *mef-2* also fully suppressed the pheromone hypersensitivity phenotype of *kin-29* mutants, but did not result in pheromone hypersensitivity on their own (Figure 2B). *hda-4(ok518)* only partly suppressed the body length and pheromone hypersensitivity phenotypes of *kin-29(oy39)* mutants, and failed to suppress the phenotypes of *kin-29(oy38)* animals (data not shown). These results suggest that KIN-29 acts by antagonizing the functions of MEF-2 and HDA-4 in the regulation of CR gene expression, body size and dauer pheromone hypersensitivity.

MEF-2 and HDA-4 act in the chemosensory neurons to suppress all kin-29 phenotypes

Previous reports indicated that MEF-2 and HDA-4 proteins were broadly expressed in multiple cell types (Dichoso *et al*, 2000; Choi *et al*, 2002). To determine whether MEF-2 and HDA-4 were expressed in the AWB and ASH chemosensory neurons, we generated *gfp*-tagged *mef-2* and *hda-4* genomic constructs. These constructs fully rescued the *mef-2*- and *hda-4*-mediated suppression, respectively, of CR::*gfp* gene expression and body size phenotypes of *kin-29* mutants (Table I and Figure 2A). GFP was localized to the nuclei of multiple sensory (including the AWB and ASH chemosensory) and non-sensory neurons, as well as in non-neuronal cells (data not shown). Nuclear localization was observed at all developmental stages, including the dauer stage and under different environmental conditions, including exposure to high concentrations of pheromone, absence of food and heat stress.

We previously showed that KIN-29 acts cell-autonomously to regulate CR gene expression and non-cell-autonomously in chemosensory neurons to regulate body size and entry into the dauer stage (Lanjuin and Sengupta, 2002). We explored the site(s) of action of MEF-2 and HDA-4 by expressing *mef-2* and *hda-4* cDNAs specifically in different cell types using cell- and tissue-specific promoters and assaying for loss of suppression of *kin-29* mutant phenotypes. Expression of *mef-2* and *hda-4* in the AWB neurons under the *lim-4* (Sagasti *et al*, 1999) or *srd-23* (Colosimo *et al*, 2004) promoters resulted in reduced *str-1*::*gfp* expression levels similar to those observed in *kin-29* single mutants, but did not lead to decreased body length (Table I and Figure 2A). However, expression of both genes under the pan-neural *unc-14* promoter (Ogura *et al*, 1997) or the *odr-4* promoter, which drive expression in most chemosensory neurons (Dwyer *et al*, 1998), was sufficient to result in loss of suppression of other *kin-29* phenotypes (Table I and Figure 2). These results indicate that similar to KIN-29, expression of MEF-2 and HDA-4 in the AWB neurons is sufficient to regulate CR gene expression, whereas expression in the chemosensory neurons is sufficient to regulate body size and entry into the dauer stage.

Table I Mutations in *mef-2* and *hda-4* suppress the reduced CR gene expression phenotype of *kin-29* mutants

Strain[a]	% expressing *str-1*::*gfp* at WT levels	*n*	*P*-values
Wild type	100	280	
kin-29(oy39)	0	218	<0.001
kin-29(oy38)	0	267	<0.001
mef-2(gv1)	100	155	
mef-2(oy65)	100	223	
hda-4(oy57)	100	189	
mef-2(oy63); kin-29(oy39)	100	204	
mef-2(oy65); kin-29(oy39)	100	158	
mef-2(oy65); kin-29(oy38)	100	188	
mef-2(gv1); kin-29(oy39)	100	209	
mef-2(gv1); kin-29(oy38)	100	194	
mef-2(gv1); kin-29(oy39); Ex[*gfp*-tagged *mef-2* genomic]	5	121	<0.001
mef-2(oy65); kin-29(oy39); Ex[*srd-23*::*mef-2*]	25	153	<0.001
mef-2(oy65); kin-29(oy39); Ex[*lim-4*::*mef-2*]	27	174	<0.001
kin-29(oy38) hda-4(oy57)	100	201	
kin-29(oy39) hda-4(oy59)	100	211	
kin-29(oy38) hda-4(ok518)	0	224	<0.001
kin-29(oy39) hda-4(ok518)	0	228	<0.001
kin-29(oy38) hda-4(oy57)/ hda-4(ok518)	96	144	
rrf-3(pk1426); kin-29(oy38) hda-4(RNAi)[b]	21	65	<0.001[c]
kin-29(oy38) hda-4(oy57); Ex[*gfp*-tagged *hda-4* genomic]	11	155	<0.001
kin-29(oy38) hda-4(oy57); Ex[*odr-4*::*hda-4*::*gfp*]	8	146	<0.001
kin-29(oy38) hda-4(oy57); Ex[*lim-4*::*hda-4*]	10	188	<0.001

Strain[a]	% expressing *sra-6*::*gfp* at WT levels	*n*	*P*-values
Wild type	100	234	
kin-29(oy38)	0	245	<0.001
mef-2(gv1)	100	153	
hda-4(oy57)	100	101	
mef-2(gv1); kin-29(oy38)	100	167	
kin-29(oy38) hda-4(oy57)	100	189	

Adult animals grown at 20°C were examined. Shown are the percentages of animals expressing *str-1*::*gfp* or *sra-6*::*gfp* in at least one AWB or ASH neuron, respectively, at wild-type levels (×50 magnification). *P*-values were determined using a two-sample *t*-test between proportions. Only significant differences ($P<0.001$), compared to wild type unless indicated otherwise, are shown.

[a]For strains carrying extrachromosomal arrays, data shown are the averages of two or more independent transgenic lines. All strains carry integrated copies of *str-1*::*gfp* or *sra-6*::*gfp*.

[b]Animals were fed with bacteria expressing *hda-4* dsRNA, starting at the L4 larval stage, and *str-1*::*gfp* expression was examined 24 h later.

[c]Data compared to *kin-29(oy38)*.

Phosphorylation of HDA-4 by KIN-29 is necessary for str-1 expression

In mammals, class II HDACs are phosphorylated at two conserved residues by upstream kinases, including CaMKII. Phosphorylated HDACs translocate from the nucleus to the cytoplasm, thereby alleviating HDAC-mediated repression of gene expression (McKinsey *et al*, 2000a, b; Kao *et al*, 2001; Chawla *et al*, 2003; Linseman *et al*, 2003). Mutations of two CaMK phosphorylation sites in HDAC5 have been shown to result in increased nuclear localization and constitutive repression of gene expression (Lu *et al*, 2000a; McKinsey *et al*, 2000a, b). Alignment of HDA-4 sequences with those of class II HDACs identified S198 in HDA-4 as analogously located to the CaMK target S259 residue in mammalian HDAC5 (Figure 3A), although no residue analogous to the S498 target amino acid could be definitively identified (data not shown). We generated full-length genomic *hda-4* genes encoding either wild-type HDA-4 or HDA-4(S198A) driven under the *hda-4* promoter. Introduction of either wild-type HDA-4 or HDA-4(S198A) fully abolished the suppression of *str-1*::*gfp* expression phenotype in *kin-29 hda-4* mutants (Table II), indicating that the HDA-4(S198A) mutant protein was capable of rescuing the *hda-4* mutant phenotype. However, we observed significant downregulation of *str-1*::*gfp* expression upon introduction of HDA-4(S198A), but not wild-type HDA-4, into wild-type or *hda-4(oy57)* animals (Table II). Both wild-type and HDA-4(S198A) retained nuclear localization in wild-type and *kin-29* mutant backgrounds (data not shown). These results suggest that phosphorylation of HDA-4 at S198 may be required to alleviate HDA-4-mediated repression of *str-1*::*gfp* gene expression, and that HDA-4(S198A) acts dominantly to repress *str-1*::*gfp* expression.

The consensus motif for SIK phosphorylation has been determined (Horike *et al*, 2003; Screaton *et al*, 2004). In HDAC5, this motif is identical to the motif targeted by CaMKs (McKinsey *et al*, 2000a; Screaton *et al*, 2004), and SIK2 can phosphorylate HDAC5 at this site *in vitro* (Screaton *et al*, 2004). We explored whether KIN-29 can also directly phosphorylate HDA-4. Functional GFP-tagged KIN-29 was immunoprecipitated from worm extracts with anti-GFP antibodies and incubated with bacterially purified HDA-4 peptides in the presence of [γ-^{32}P]ATP. As shown previously, KIN-29 undergoes autophosphorylation at an unidentified site(s) (Maduzia *et al*, 2005) (Figure 3B). We also observed phosphorylation of HDA-4 by KIN-29. Consistent with the genetic data presented above, phosphorylation of HDA-4 was abrogated by the S198A mutation (Figure 3B). We did not detect phosphorylation of bacterially produced MEF-2 under similar conditions (data not shown). These results suggest that KIN-29 may directly target HDA-4 at the S198 residue.

Constitutive Ca^{2+} signaling can partly bypass the requirement for KIN-29 in the regulation of CR gene expression

As Ca^{2+} signaling has been shown to regulate MEF2/HDAC functions, we explored the possibility that Ca^{2+} signaling also plays a role in regulating *str-1* expression via the KIN-29/MEF-2/HDA-4-mediated pathway. Loss-of-function mutations in the voltage-gated Ca^{2+} channel genes *unc-36* and *egl-19* (Schafer *et al*, 1996; Lee *et al*, 1997), and the *unc-43* CaMKII gene (Reiner *et al*, 1999) did not result in altered *str-1*::*gfp* expression in either wild-type or *kin-29* mutant backgrounds (Table III). However, a *unc-43(gf)* and *egl-19(gf)* allele partially suppressed the *str-1*::*gfp* gene expression phenotype in *kin-29* mutants (Table III). No gross alterations were observed in *str-1*::*gfp* gene expression in a wild-type background. The *gf* mutation in *unc-43* is predicted to result in a Ca^{2+}-independent, constitutively activated CaMKII enzyme (Reiner *et al*, 1999), whereas the *gf* mutation in *egl-19* is

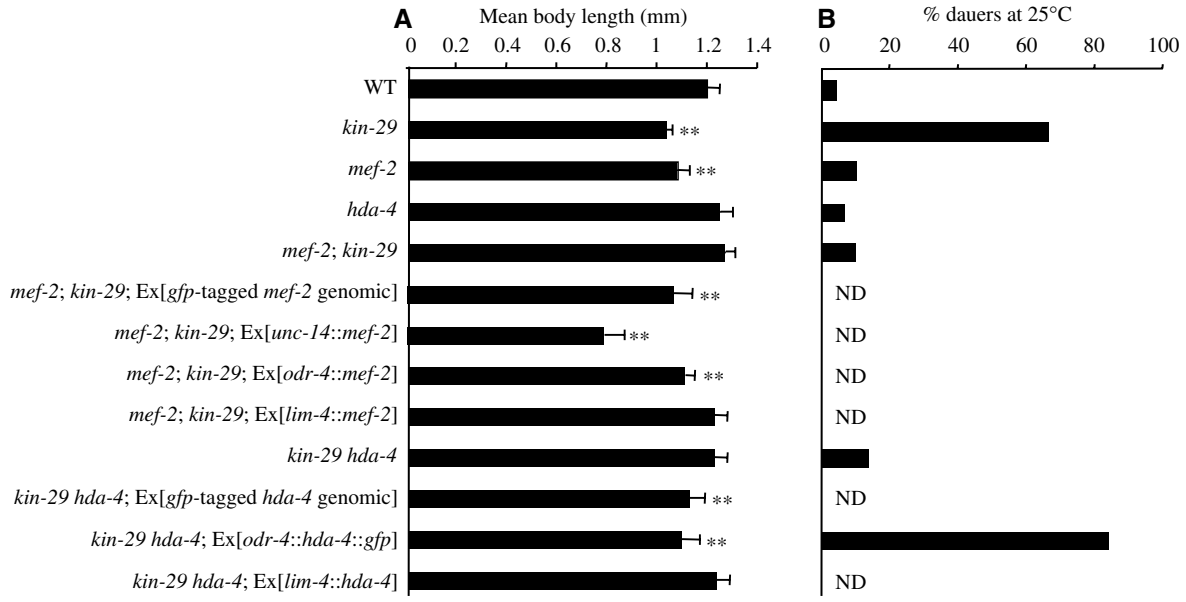

Figure 2 *lf* mutations in *mef-2* and *hda-4* suppress the decreased body size and pheromone hypersensitivity phenotypes of *kin-29* mutants. (**A, B**) Body lengths (A) and dauer pheromone hypersensitivity (B) of animals of the indicated genotypes. Alleles used were *kin-29(oy38)*, *kin-29(oy39)*, *mef-2(gv1)*, *mef-2(oy65)* and *hda-4(oy57)*. No significant differences were observed between animals carrying different alleles of a gene. For strains carrying extrachromosomal arrays, data shown are the average of two or more independent transgenic lines, which were not significantly different from each other using a two-sample *t*-test. For dauer assays, data shown are from a single experiment with all strains assayed in parallel, with the exception of strains carrying transgenic arrays. These were tested independently together with animals that have lost the array as an internal control. All strains contain stably integrated *str-1*::*gfp* fusion genes. Error bars denote the s.d.; $n = 35$–48 (A) and 200–400 (B). Double asterisks denote values that are different from wild type at $P < 0.001$. n.d., not done.

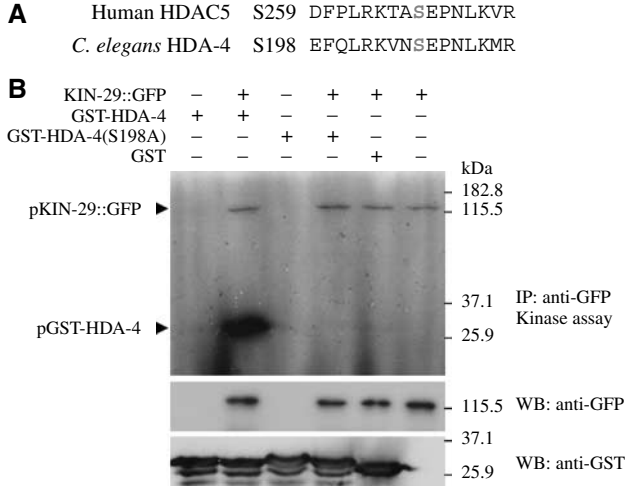

Figure 3 KIN-29 phosphorylates HDA-4 at residue S198 *in vitro*. (**A**) Alignment of the CaMK/SIK phosphorylation target sequences in HDAC5 and HDA-4. The targeted S259 and S198 residues in HDAC5 and HDA-4, respectively, are in red. (**B**) *In vitro* kinase assay. Phosphorylated proteins (pKIN-29::GFP and pGST-HDA-4) were detected by autoradiography. Substrates used were bacterially purified wild-type or S198A mutated HDA-4 (residues 191–204) fused to GST or GST alone. Western blotting was performed with anti-GFP and anti-GST antibodies to detect KIN-29::GFP and GST fusion proteins, respectively.

predicted to prolong depolarization and result in sustained Ca^{2+} influx (Lee *et al*, 1997). Interestingly, unlike in *mef-2*; *kin-29* or *kin-29 hda-4* double mutants, in which upregulation of *str-1*::*gfp* expression was evident at all developmental stages, upregulation of gene expression was observed primarily at later larval stages and in adults (1% of L1, 21% of L2, 67% of L3 and 82% of *unc-43(gf)*; *kin-29(oy39)* L4 larvae and adults exhibited stronger *str-1*::*gfp* expression; $n = 100$). Moreover, *unc-43(gf)* failed to suppress the dauer pheromone hypersensitivity phenotype and the *sra-6*::*gfp* expression downregulation phenotype of *kin-29* mutants (Supplementary Table 2). These results indicate that increased or constitutive Ca^{2+} signaling is able to partially bypass the requirement for a subset of KIN-29 functions in a subset of cells at later developmental stages. HDA-4(S198A) reduced the *unc-43(gf)*-mediated upregulation of *str-1*::*gfp* expression in *kin-29* mutants (Table III), suggesting, but not proving, that constitutively activated UNC-43 may bypass KIN-29 by phosphorylating HDA-4 at the S198 residue.

Calcineurin acts in parallel to the KIN-29-regulated pathway to modulate CR gene expression

The Ca^{2+}-dependent Ser/Thr phosphatase calcineurin dephosphorylates and antagonizes SIK-mediated phosphorylation of the CREB coactivator TORC2 in the regulation of gene expression in cultured cells (Screaton *et al*, 2004). Calcineurin also activates MEF2 via desphosphorylation of a specific residue, coupled with suppression of sumoylation at a neighboring residue (Mao and Wiedmann, 1999; Wu *et al*, 2001; Gregoire *et al*, 2006; Kang *et al*, 2006; Shalizi *et al*, 2006). To determine the role of calcineurin in KIN-29/MEF-2-regulated CR gene expression, we examined *str-1*::*gfp* expression in animals mutant for the *cnb-1* calcineurin regulatory and the *tax-6* calcineurin catalytic subunit genes (Bandyopadhyay *et al*, 2002; Kuhara *et al*, 2002). However, *lf* mutations in either gene did not affect *str-1*::*gfp* expression (Table IV). We next determined whether mutations in *tax-6*/*cnb-1* could bypass the requirement for KIN-29 in the regulation of *str-1*::*gfp* expression. *lf* mutations in both *tax-6* and

Table II The HDA-4(S198A) mutation constitutively represses *str-1*::*gfp* expression

Strain[a,b]	% expressing *str-1*::*gfp* at WT levels	*n*	*P*-values
Wild type	100	120	
kin-29(oy38)	0	120	<0.001
hda-4(oy57)	100	109	
Ex[*hda-4*::*hda-4*]	100	110	
Ex[*hda-4*::*hda-4* (S198A)]	18	112	<0.001
kin-29(oy38); Ex[*hda-4*::*hda-4*]	0	102	<0.001
kin-29(oy38); Ex[*hda-4*::*hda-4* (S198A)]	0	105	<0.001
hda-4(oy57); Ex[*hda-4*::*hda-4*]	100	117	
hda-4(oy57); Ex[*hda-4*::*hda-4* (S198A)]	17	115	<0.001
kin-29(oy38) hda-4(oy57)	100	119	
kin-29(oy38) hda-4(oy57); Ex[*hda-4*::*hda-4*]	10	108	<0.001
kin-29(oy38) hda-4(oy57); Ex[*hda-4*::*hda-4* (S198A)]	11	110	<0.001

Adult animals grown at 20°C were examined. Shown are the percentages of animals expressing *str-1*::*gfp* in at least one AWB neuron at wild-type levels (×50 magnification). *P*-values were determined using a two-sample *t*-test between proportions. Only significant differences ($P<0.001$), compared to wild type, are shown.
[a]All strains contain stably integrated *str-1*::*gfp* fusion genes.
[b]For strains carrying extrachromosomal arrays, data shown are the averages of two or more independent transgenic lines. All *hda-4* genomic sequences are tagged with *gfp* coding sequences.

Table III Constitutive Ca^{2+} signaling partially bypasses the requirement for KIN-29 in regulating *str-1*::*gfp* expression

Strain[a]	% expressing *str-1*::*gfp* at WT levels	*n*	*P*-values
Wild type	100	200	
kin-29(oy39)	0	200	<0.001
kin-29(oy38)	0	200	<0.001
CaMKII			
unc-43(e408)lf	100	134	
unc-43(n498)gf	100	153	
unc-43(e408)lf; kin-29(oy39)	0	112	[b]
unc-43(e408)lf; kin-29(oy38)	0	140	[b]
unc-43(n498)gf; kin-29(oy39)	66	167	<0.001[b]
unc-43(n498)gf; kin-29(oy38)	72	122	<0.001[b]
unc-43(n498)gf; kin-29(oy38); Ex[593 bp *str-1*::*gfp*]	78	115	<0.001[b]
unc-43(n498)gf; kin-29(oy38); Ex[*hda-4*::*hda-4* (S198A)]	34	135	<0.001[b]
Ca^{2+} channels			
unc-36(e251)	100	120	
egl-19(n582)lf	100	100	
egl-19(n2368)gf	100	130	
unc-36(e251); kin-29(oy39)	0	125	[b]
egl-19(n582)lf; kin-29(oy39)	0	150	[b]
egl-19(n2368)gf; kin-29(oy39)	56	34	<0.001[b]

Data shown are from adult animals grown at 20°C. Shown are the percentages of animals expressing *str-1*::*gfp* in at least one AWB neuron at wild-type levels (×50 magnification). *P*-values were determined using a two-sample *t*-test between proportions. Only significant differences ($P<0.001$), compared to wild type or *kin-29* as indicated, are shown.
[a]All strains contain stably integrated copies of *str-1*::*gfp* with the exception of *unc-43(n498)gf; kin-29(oy38)*, which carries an extrachromosomal array of a construct containing 593-bp *str-1* promoter sequences fused to *gfp*. The *hda-4* genomic sequence is tagged with a *gfp*-coding sequence.
[b]Data compared to *kin-29(oy38)* and *kin-29(oy39)*.

cnb-1 partially suppressed the *str-1*::*gfp* expression phenotypes of both *kin-29(oy38)* null and *kin-29(oy39)* missense mutants (Table IV), suggesting that calcineurin antagonizes KIN-29 to regulate CR gene expression. Calcineurin targets the S408/S444 residue in MEF2A/D for dephosphorylation, which, in turn, has been shown to be targeted for phosphorylation by the CDK5 Ser/Thr kinase (Gong *et al*, 2003; Flavell *et al*, 2006; Shalizi *et al*, 2006). Mutation of the corresponding conserved residue (S321) in MEF-2, or *lf* mutations in the *cdk-5* ortholog, did not affect MEF-2 functions (Table IV), suggesting that calcineurin may target another site in MEF-2 or target alternate regulatory proteins.

As sumoylation has also been implicated in the regulation of MEF2 function, we next determined whether sumoylation plays a role in modulating MEF-2 activity. The residue targeted for sumoylation in MEF2 proteins is conserved in MEF-2. However, an MEF-2(K316R) mutation did not affect *str-1*::*gfp* expression in wild-type animals, and fully rescued the *mef-2*-mediated suppression of the *str-1*::*gfp* expression phenotype in *kin-29* mutants (Table IV). Consistent with this result, *lf* mutations in *smo-1*, the sole SUMO ortholog encoded by the *C. elegans* genome (Broday *et al*, 2004), also did not alter *str-1*::*gfp* expression levels (Table IV). This result suggests that sumoylation does not play a major role in this gene regulatory process.

MEF-2 directly binds CR upstream regulatory sequences to regulate gene expression

We determined whether MEF-2 regulates CR gene expression directly or indirectly, by carrying out a deletion analysis of the *str-1* upstream regulatory sequences to define KIN-29- and MEF-2-regulated sites (Figure 4A). Previously, 1.8 kb of *str-1* upstream sequences were shown to drive KIN-29-regulated *gfp* expression in the AWB neurons (Troemel *et al*, 1997; Lanjuin and Sengupta, 2002). We narrowed the required minimal sequences to 593 bp upstream of the start codon. Further deletion analyses within these minimal sequences defined two regions required for correct regulation of *str-1*::*gfp* expression. Although ~200 bp sequences upstream of the start codon drove *gfp* expression in the AWB neurons, expression driven by these sequences was no longer regulated by KIN-29 (Figure 4A). Deletion of a 29 bp sequence located −237 to −208 relative to the start codon also fully abolished KIN-29-mediated regulation of expression in the context of either the 1.8 kb or the 593 bp minimal *str-1* regulatory sequences (Figure 4A). These results suggest that the element required for activation of *str-1*::*gfp* expression in the AWB neurons is distinct from the element required for KIN-29-mediated modulation of gene expression.

Target sequences recognized by MEF2 family members, including *C. elegans* MEF-2, have been well characterized (Gossett *et al*, 1989; Pollock and Treisman, 1991; Dichoso *et al*, 2000). We identified a site related to the MEF-2-binding element within the 29 bp sequence required for KIN-29-mediated regulation of *str-1*::*gfp* expression. Mutating the core of the MEF-2-binding site alone resulted in upregulation

Table IV *lf* mutations in calcineurin suppress *kin-29(lf)* mutations

Strain[a,b]	% expressing *str-1*::*gfp* at WT levels	*n*	*P*-values
Wild type	100	250	
kin-29(oy39)	0	250	<0.001
kin-29(oy38)	0	250	<0.001
Calcineurin			
cnb-1(jh103)	100	206	
tax-6(p675)	100	233	
cnb-1(jh103); kin-29(oy39)	27	180	<0.001[c]
cnb-1(jh103); kin-29(oy38)	36	145	<0.001[c]
tax-6(p675); kin-29(oy39)	32	155	<0.001[c]
SUMO/CDK5			
smo-1(ok359)	100	102	
cdk-5(ok626)	100	155	
smo-1(ok359); kin-29(oy39)	0	108	[c]
cdk-5(ok626); kin-29(oy39)	0	167	[c]
mef-2(oy65); kin-29(oy39)	100	240	
mef-2(oy65); kin-29(oy39); Ex[*mef-2*::*mef-2* genomic]	9	195	<0.001
mef-2(oy65); kin-29(oy39); Ex[*mef-2*::*mef-2* (K316R)]	6	164	<0.001
mef-2(oy65); kin-29(oy39); Ex[*mef-2*::*mef-2* (S321A)]	6	200	<0.001
Ex[*mef-2*::*mef-2* genomic]	100	145	
Ex[*mef-2*::*mef-2* (K316R)]	100	134	

Adult animals grown at 20°C were examined. *P*-values were determined using a two-sample *t*-test between proportions. Only significant differences ($P<0.001$), compared to wild type or *kin-29* as indicated, are shown.
[a]All strains contain stably integrated *str-1*::*gfp* fusion genes.
[b]For strains carrying extrachromosomal arrays, data shown are the average of three or more independent transgenic lines.
[c]Data compared to *kin-29(oy38)* and *kin-29(oy39)*.

of *str-1*::*gfp* expression levels in *kin-29* mutants (Figure 4B). Sequence analysis of the ~3.5 kb *sra-6* upstream regulatory sequences also identified a single predicted MEF-2-binding element, which, when deleted, resulted in upregulation of *sra-6*::*gfp* expression in *kin-29* mutants (Supplementary Figure 1). No effect was observed on *str-1*::*gfp* or *sra-6*::*gfp* expression upon mutation of the predicted MEF-2 site in a wild-type background. To confirm that MEF-2 directly regulates *str-1* expression, we carried out an electrophoretic mobility shift assay (EMSA) using epitope-tagged MEF-2 protein and the MEF-2 recognition site derived from *str-1* upstream sequences. MEF-2 specifically bound these sequences *in vitro*, as evidenced by the retarded mobility of the DNA–protein complex (Figure 4C). No binding was observed to a mutated MEF-2-binding site. These results suggest that MEF-2 directly binds CR gene regulatory sequences to modulate gene expression.

Insertion of MEF-2-binding site sequences is sufficient to confer KIN-29-mediated regulation on CR genes

We had previously shown that expression of only a subset of CR genes was altered in *kin-29* mutants (Lanjuin and Sengupta, 2002). In recent work, we identified additional CR genes expressed in the AWB and other chemosensory neurons (Colosimo *et al*, 2004). However, expression of *srsx-3*, *srd-23* and *sru-38* CR::*gfp* fusion genes was unaltered in *kin-29(oy38)* animals, and we did not identify predicted MEF-2-binding elements in their upstream regulatory sequences (Figure 5A and C, Supplementary Table 1). We wondered whether insertion of an MEF-2-binding site into CR gene regulatory sequences would be sufficient to confer KIN-29-mediated regulation of gene expression. As the element necessary to drive expression in the AWB neurons and the MEF-2 recognition element were located in close proximity in the *str-1* regulatory sequences, we inserted a 26 bp sequence containing the predicted MEF-2-binding site derived from *str-1* into the regulatory sequences of the *srsx-3* gene in close proximity to an element identified as necessary to drive expression in the AWB and AWC neurons (Nokes *et al*, in preparation) (Figure 5B). Insertion of these sequences resulted in significant downregulation of *gfp* expression in both the AWB and AWC neurons in a *kin-29* mutant background (Figure 5B and C). This downregulation was suppressed in *mef-2*; *kin-29* double mutants (Figure 5C). However, insertion of the MEF-2 sequences further upstream failed to confer KIN-29-mediated regulation of expression (Figure 5C). These results indicate that the identified MEF-2-binding site is both necessary and sufficient to confer KIN-29-mediated regulation onto CR genes, but that the relative location of this site with respect to additional regulatory sites is likely to be critical for this regulation.

Identification of additional KIN-29/MEF-2-regulated CR genes

To identify additional KIN-29/MEF-2-regulated CR genes, we searched the genome for candidate CRs that contain a predicted MEF-2-binding site, as well as a cell-specific regulatory element in close proximity. Although elements driving gene expression in specific chemosensory neuron types are largely unknown, recently, an E-box motif was identified as being necessary to drive CR gene expression in the ADL chemosensory neurons (McCarroll *et al*, 2005). However, none of the identified ADL-expressed CR genes contained the predicted MEF-2-binding sites in their upstream regulatory sequences, and were not regulated by KIN-29 (Supplementary Table 1). We identified three additional CR genes (*srh-60*, *srz-24*, *srh-234*) that contained the E-box motif as well as a single predicted MEF-2-binding site in their upstream regulatory sequences. The E-box and the predicted MEF-2 site were located in close proximity upstream of the *srz-24* and *srh-234*, but not of the *srh-60* genes (Supplementary Table 1). All three gene regulatory sequences drove *gfp* expression in the ADL neurons (Figure 5D and Supplementary Table 1). Expression driven by the *srh-234* but not the *srz-24* or *srh-60* regulatory sequences was strongly downregulated in *kin-29* mutants (Figure 5D and E and Supplementary Table 1). This downregulation of expression was fully suppressed by mutations in *mef-2* (Figure 5E). These results further suggest that the location of the predicted MEF-2 site relative to other regulatory elements is one important parameter conferring KIN-29/MEF-2-mediated regulation of gene expression onto CR genes.

Discussion

The KIN-29 SIK regulates gene expression via MEF-2/HDAC

Results presented in this work indicate that the KIN-29 SIK targets the HDA-4 class II HDAC for phosphorylation, and

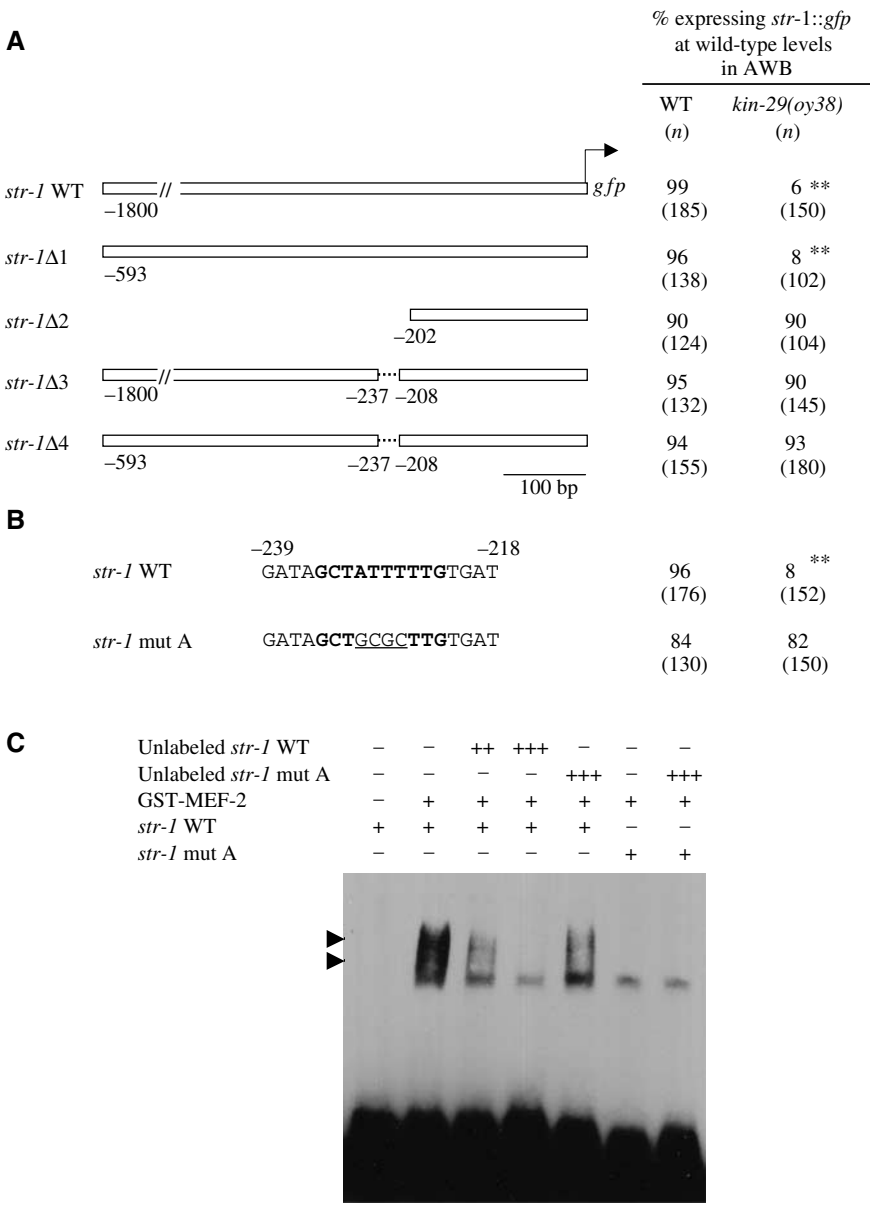

Figure 4 MEF-2 binds directly to *str-1* regulatory sequences to regulate gene expression. (**A**) Expression of *gfp* driven by the indicated *str-1* regulatory sequences in wild-type and *kin-29(oy38)* mutant animals. Numbers shown are the average of at least two independent transgenic lines carrying each construct. Animals were examined at ×50 magnification. Double asterisks denote values different from wild type at $P<0.001$ using a two-sample *t*-test between proportions. (**B**) A predicted MEF-2-binding site is in bold. Mutations generated in this site are underlined. Numbers shown are the average of two independent transgenic lines carrying the mutated MEF-2 site introduced into the 593 bp *str-1* promoter. Animals were examined at ×50 magnification. Double asterisks denote values different from wild type at $P<0.001$ using a two-sample *t*-test between proportions. (**C**) The interaction of bacterially expressed GST-MEF-2 fusion protein with a predicted MEF-2-binding site from the *str-1* promoter was examined using EMSA. Two strongly shifted bands are shown (arrowheads), presumably representing homodimeric or oligomeric complexes, as described previously (Dichoso *et al*, 2000). Concentrations of sequences used are as follows: + = 10 fmol; ++ = 5 pmol; +++ = 50 pmol.

thereby alleviates MEF-2/HDA-4-mediated repression of CR gene expression in chemosensory neurons. Although AMPK has been suggested to regulate skeletal muscle gene expression via MEF2 and associated proteins in response to exercise (Al-Khalili *et al*, 2004; Holmes *et al*, 2005), to our knowledge, this work is the first to demonstrate that an SIK regulates MEF2/HDAC functions in the nervous system, and that this regulation is biologically relevant *in vivo*.

KIN-29 may target HDA-4 at the S198 residue, and an HDA-4(S198A) mutation results in constitutive repression of CR gene expression. Thus, we suggest that in *kin-29* mutants, HDA-4 is constitutively dephosphorylated, resulting in localized chromatin condensation (reviewed in Yang and Gregoire, 2005) (Figure 6A and B). This alteration may prevent activation of gene expression by protein(s) binding to sites located in close proximity, such as activators required to drive *str-1* expression in the AWB neurons. Upon phosphorylation by KIN-29 and/or CaMKs such as CaMKII, this repression is alleviated. In mammalian cells, phosphorylation of HDACs results in translocation of HDACs to the cytoplasm (McKinsey *et al*, 2000a; Kao *et al*, 2001; Chawla *et al*, 2003). Moreover, phosphorylation of TORC2 by SIK also results in

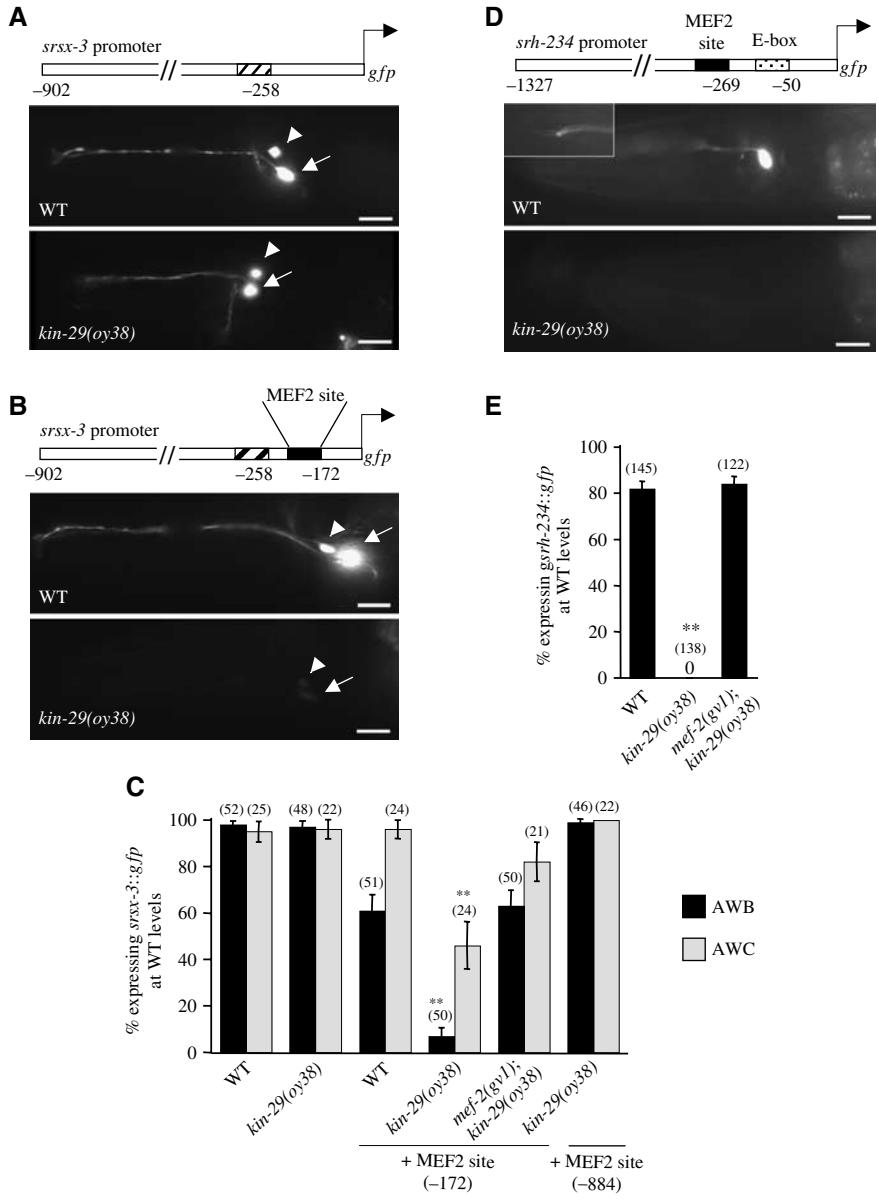

Figure 5 Location of an MEF-2 element in close proximity to cell-specific regulatory elements confers KIN-29-mediated regulation onto CR genes. (**A, B**) Expression of *gfp* driven by 902 bp *srsx-3* regulatory sequences without (A) or with (B) an inserted *mef-2* element (filled box) in AWB (arrowhead) and AWC (arrow) neurons of wild-type (top) and *kin-29(oy38)* mutant (bottom) animals. Hatched box indicates sequences required to drive *gfp* expression in the AWB and AWC neurons. Numbers indicate the position of each element in the *srsx-3* regulatory sequences with respect to the start codon of *srsx-3*. Lateral view: anterior is on the left. Scale, 15 μm. (**C**) The percentage of AWB or AWC neurons of the indicated genotypes expressing *srsx- 3*::*gfp* at wild-type levels is indicated. Numbers indicate the position of the inserted MEF-2 site in the *srsx-3* upstream regulatory sequences with respect to the start codon. Adult animals grown at 20°C were examined at × 400 magnification. Numbers in parentheses indicate numbers of animals examined. Error bars denote the SEP. Double asterisks indicate values different from the matched wild-type controls at $P<0.001$ using a two-sample *t*-test between proportions. (**D**) Expression of *srh-234*::*gfp* in an ADL neuron of wild-type (top) or *kin-29(oy38)* mutant animals. The inset shows the characteristic doublet sensory cilia of an ADL neuron. Filled box indicates the *mef-2* element; stippled box indicates the E-box shown to be required to drive CR gene expression in the ADL neurons (McCarroll *et al*, 2005). Anterior is on the left. Scale, 15 μm. (**E**) Percentage of animals of the indicated genotypes expressing *srh-234*::*gfp* in at least one ADL neuron. Adult animals grown at 20°C were examined at × 50 magnification. Error bars denote the SEP. Double asterisks indicate values different from wild type at $P<0.001$ using a two-sample *t*-test between proportions.

cytoplasmic sequestering (Screaton *et al*, 2004; Koo *et al*, 2005). Unlike in other cultured cell types, we did not observe phosphorylation state-dependent nucleoplasmic shuttling of either HDA-4 or MEF-2. Similarly, we also observed cytoplasmic localization of KIN-29 under all conditions examined (Lanjuin and Sengupta, 2002). Thus, although in both cases we are unable to exclude transient shuttling events or shuttling of a subset of molecules, it remains formally possible that KIN-29 acts via a downstream kinase. This kinase is unlikely to be solely UNC-43 CaMKII, as *lf* mutations in *unc-43* have no effects on CR gene expression.

Our results also reveal a new role of MEF2 proteins in the regulation of gene expression. In neurons and other cell types, phosphorylation of class II HDACs has been shown to result in the dissociation of HDACs from MEF2, thereby allowing MEF2 to promote gene expression in association

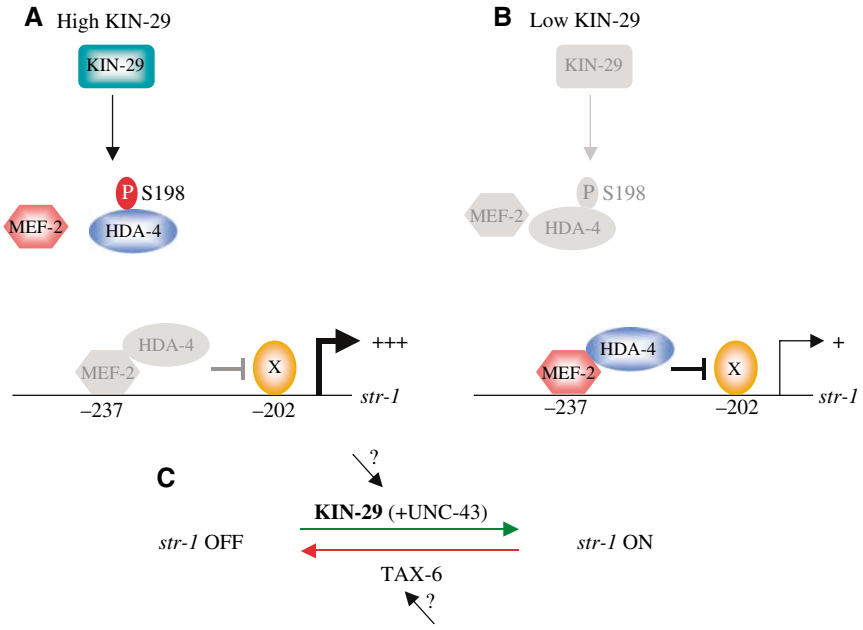

Figure 6 A model for regulation of *str-1* CR expression by KIN-29/MEF-2/HDA-4. (**A**) Under conditions of high KIN-29 activity, HDA-4 is phosphorylated by KIN-29 at S198, inhibiting MEF-2/HDA-4-mediated repression of gene expression. X represents a transcriptional activator that binds to sequences within −200 bp of the start codon to drive *str-1* expression specifically in the AWB neurons. The gene activation functions of this protein may be inhibited by DNA-bound MEF-2/HDA-4. (**B**) Under conditions of low KIN-29 activity, the majority of HDA-4 molecules are unphosphorylated and interact with MEF-2 to repress *str-1* expression. (**C**) *str-1* CR gene expression is regulated by the antagonistic actions of the KIN-29 kinase and the TAX-6 calcineurin phosphatase. Phosphorylation of HDA-4 by KIN-29 and other kinases such as UNC-43 CaMKII results in activation of gene expression, whereas dephosphorylation by TAX-6 results in repression of gene expression. The inputs into KIN-29 and TAX-6 functions are not yet known.

with transcriptional coactivators (Lu *et al*, 2000a). However, we did not detect a requirement for MEF-2 in the positive regulation of CR gene expression, such that levels of *str-1* expression are not appreciably altered in an *mef-2* mutant background. MEF-2 and HDA-4 have been shown to physically interact (Choi *et al*, 2002), and we find that mutations in these genes act similarly to suppress *kin-29* phenotypes. Thus, MEF-2 and HDA-4 likely act in a complex to regulate *str-1* gene expression. These observations suggest that in the absence of KIN-29 function, direct DNA binding by MEF-2 may be required to recruit HDA-4 to the *str-1* regulatory sequences, but that unlike in other systems, MEF-2 is not itself required to drive *str-1* expression.

What is the role of calcineurin in the regulation of CR gene expression via KIN-29/MEF-2? In cultured cells, calcineurin antagonizes SIK to regulate TORC2 function by regulating its subcellular localization (Screaton *et al*, 2004). Thus, the balance between phosphorylation and dephosphorylation of TORC2 by SIK and calcineurin, respectively, regulates the gene activation properties of TORC2. A similar mechanism may operate in the regulation of CR gene expression by the KIN-29 pathway (Figure 6C). In this case, phosphorylation of the MEF-2/HDA-4 complex results in gene expression, whereas dephosphorylation results in repression. In the simplest model, we suggest that the MEF-2/HDA-4 complex is targeted by KIN-29 as well as by an additional kinase(s) such as UNC-43 CaMKII, leading to inhibition of its repressive functions. In the absence of KIN-29-mediated phosphorylation, but in the presence of calcineurin, the MEF-2/HDA-4 complex represses gene expression. Loss of calcineurin function may partly compensate for the decreased levels of phosphorylation in *kin-29* mutants to alleviate this repression, thereby leading to partial suppression of the *kin-29* mutant phenotype. The site(s) targeted by calcineurin in the MEF-2/HDA-4 complex in the AWB neurons is not yet known.

Multiple cis-regulatory elements drive correct levels of CR gene expression

In this analysis, we identified an element in the *str-1* regulatory sequences that is required to drive *str-1*::*gfp* expression in the AWB neurons developmentally, as well as a distinct element required to interact with MEF-2. The MEF-2-interacting site is both necessary and sufficient to modulate expression levels, but does not itself dictate spatial expression patterns. Introduction of a single MEF-2-binding site into a KIN-29-independent CR gene regulatory sequence was sufficient to confer KIN-29-mediated regulation, although the relative position of the MEF-2 site is important. These results suggest that CR gene expression is directed by multiple regulatory modules, where a module consists of a *cis*-regulatory site and the *trans*-acting factor(s) interacting with the site (Yuh and Davidson, 1996; Markstein *et al*, 2004). Each of these modules may be regulated by different developmental or environmental conditions, thereby conferring appropriate regulation of CR gene expression. However, it remains possible that in some CR gene regulatory sequences, multiple signaling pathways converge onto a single module.

Physiological consequences of CR gene regulation by the KIN-29/MEF-2/HDA-4 pathway

In contrast to more complex nervous systems, the *C. elegans* nervous system is relatively shallow, consisting of only one or a few layers of interneurons between peripheral sensory

neurons and motor neurons that drive locomotion (White *et al*, 1986). We and others have previously suggested that perhaps to compensate for the relative simplicity of its nervous system structure, peripheral sensory neurons carry out complex functions in *C. elegans*, thereby contributing extensively to behavioral and developmental plasticity (Peckol *et al*, 2001; Lanjuin and Sengupta, 2002; Clark *et al*, 2006). Thus, modulation of CR gene expression via integration of external and internal cues may provide a simple mechanism for *C. elegans* to rapidly alter its physiological responses to changing conditions.

We show that expression of *kin-29*, *hda-4* and *mef-2* in the chemosensory neurons is sufficient not only to rescue the CR gene expression defects, but also to rescue the body size and dauer developmental defects (Lanjuin and Sengupta, 2002; this work). Correct acquisition and transduction of both external and internal sensory cues have been shown to be critical for the regulation of these physiological processes (Albert *et al*, 1981; Golden and Riddle, 1984; Kimura *et al*, 1997; Sze *et al*, 2000; Fujiwara *et al*, 2002; Lanjuin and Sengupta, 2002). The SIK family of kinases mediates feedback-mediated regulation of gluconeogenic gene expression in response to fasting or feeding, and has also been implicated in the regulation of insulin signaling in adipocytes (Horike *et al*, 2003; Koo *et al*, 2005). Moreover, the activity of AMPK in the hypothalamus in response to internal metabolic state regulates food intake and body weight (Minokoshi *et al*, 2004). It is thus tempting to speculate that the KIN-29 pathway may also respond to internal metabolic state, so as to appropriately modulate sensory neuron gene expression. The subset of sensory genes regulated by KIN-29 may play particularly critical roles in recognizing and responding to biologically relevant chemicals, and in regulating sensory neuron function and food intake. We note that *kin-29*, *mef-2* and *hda-4* are also expressed in multiple additional neuronal and non-neuronal cell types (Dichoso *et al*, 2000; Choi *et al*, 2002; Lanjuin and Sengupta, 2002), and are likely to act in other pathways. It will be interesting to determine whether SIKs also act via regulation of MEF2/HDAC protein functions to regulate gene expression in the nervous system of other organisms. Unlike SIK, KIN-29 may not be activated via phosphorylation by upstream kinases such as LKB1 (Lizcano *et al*, 2004), as mutations in the predicted LKB1 target sites did not affect KIN-29 functions (A Lanjuin and P Sengupta, unpublished observations). Thus, we expect that these molecules regulate gene expression in response to external signals via a plethora of distinct mechanisms in different cell types *in vivo*, thereby allowing a conserved signaling module to be utilized in multiple contexts for different gene regulatory functions.

Materials and methods

Strains

Worms were grown as previously described (Brenner, 1974). Double and triple mutant strains were constructed using standard methods and confirmed via PCR, sequencing or complementation tests.

Isolation, mapping and cloning of mef-2 and hda-4

A *kin-29(oy39)* mutant strain carrying integrated copies of *str-1*::*gfp* (*kyIs104*) was mutagenized with EMS. *mef-2(oy65)*, *mef-2(oy63)* and *hda-4(oy59)* were isolated as suppressors of the reduced *str-1*::*gfp* expression phenotype of *kin-29(oy39)* mutants in a screen of ~25 000 haploid genomes. *hda-4(oy57)* was isolated as a spontaneous suppressor of *kin-29(oy38)*. Mutations were mapped using standard methods, and mutant phenotypes rescued by wild-type genomic sequences. The molecular identities of the mutations were determined by sequencing. Suppressor strains were outcrossed 2–4 times before characterization.

Expression constructs and generation of transgenic animals

Promoter::*gfp* constructs were generated by amplifying upstream genomic sequences, which were then cloned into the pPD95.77 expression vector (gift of A Fire) or fused to *gfp*-coding sequences by PCR (Hobert, 2002). Site-directed mutageneses were carried out with the QuikChange site-directed mutagenesis kit (Stratagene) and confirmed by sequencing. For each promoter::*gfp* construct, expression from the same extrachromosomal array was examined in wild-type and *kin-29* mutant animals. *mef-2* and *hda-4* cDNAs were kind gifts from J Ahnn. Details of constructs are available upon request. Transgenic animals were generated using the *unc-122*::*dsRed* or pRF4 co-injection markers.

Detection of endogenous str-1 transcripts by RT–PCR

Total RNA isolated from mixed-stage populations of animals was reverse-transcribed using an oligo-d(T) primer. Primers specific for *str-1*- and *odr-10*-coding sequences were then used to amplify *str-1* and *odr-10* sequences, respectively, in the same amplification reaction. The amplified PCR products were electrophoresed and quantitated using Molecular Analyst (BioRad) software. Levels of *odr-10* expression are not affected by mutations in *kin-29* (Lanjuin and Sengupta, 2002).

Identification of MEF-2-binding sites upstream of additional CR genes

Deletion and site-directed mutagenesis of *str-1* and *sra-6* upstream sequences identified a DNA motif highly similar to the mammalian consensus of MEF2 ($CTA(A/T)_4TA(G/A)$) (Gossett *et al*, 1989; Pollock and Treisman, 1991; Wasserman and Fickett, 1998) that was both necessary and sufficient to confer MEF2-mediated gene regulation. We searched for MEF-2-binding sites in 1.5 kb upstream sequences of CRs containing the E-box motif (McCarroll *et al*, 2005) using a simple matrix-based motif detection program, Possum (http://zlab.bu.edu/~mfrith/possum/). The matrix used for the search was generated using the mammalian consensus MEF2 site and the MEF-2 sites experimentally identified in the *str-1* and *sra-6* promoters, together with the E-box motif. Score threshold was set at 5.

RNA interference of hda-4

RNAi was performed in the *rrf-3(pk1426)* background to enhance neuronal RNAi (Simmer *et al*, 2003). L4 larval stage *rrf-3*; *kin-29* double mutant animals carrying stably integrated *str-1*::*gfp* transgenes were placed on an NGM agar plate containing 1 mM IPTG and seeded with bacterial food, producing dsRNA directed against the *hda-4* gene. *str-1*::*gfp* expression was quantitated after 24 h. The L4440 empty vector was used as a control.

Electrophoretic mobility shift assay

To obtain purified MEF-2 protein, a full-length *mef-2* cDNA was cloned into pGEX4T-1 (a kind gift from J Ahnn) and expressed in JM109 *Escherichia coli* cells. The GST-MEF-2 fusion protein was purified on a glutathione–Sepharose column following manufacturer's recommended protocols (Amersham Biosciences). EMSA was performed by incubating 1–2 μg GST-MEF-2 fusion protein with biotin-labeled and/or unlabeled wild-type or mutant sequences. Samples were electrophoresed on a 6% native polyacrylamide gel and visualized by chemiluminescence (Pierce). Sequences used were the following: *str-1* wild type: 5′-GATAGCTATTTTTGTGAT: *str-1* mut A: 5′-GATAGCTGCGCTTGTGAT. Mutated bases are underlined.

Kinase assay

Worm lysates from a mixed-stage population of wild-type animals carrying a stably integrated functional *gfp*-tagged *kin-29* transgene (*oyIs41*) were incubated with rabbit polyclonal anti-GFP antibody (Clontech) and immunoprecipitated with protein A Sepharose 4B beads (Zymed). Immune complexes were mixed with or without substrates and divided into two aliquots. One aliquot was analyzed by Western blotting using anti-GFP and anti-GST antibodies (Amersham). The second aliquot was analyzed for KIN-29 kinase

activity by incubation with 10 μCi of [γ-^{32}P]ATP (3000 μCi/mmol). Samples were electrophoresed on an 11% denaturing polyacrylamide gel and visualized by autoradiography. Substrates used were GST-HDA-4 (aa 191–204), GST-HDA-4(S198A) (aa 191–204) or GST alone. GST fusion proteins were purified from bacteria as described above.

Examination of CR gene expression, body length and pheromone hypersensitivity phenotypes

All animals were grown at 20°C for one or two generations in well-fed conditions, using HB101 as a food source before analyses. Levels of CR promoter::*gfp* expression were examined in animals under ×50 or ×400 magnification using a dissection or compound microscope equipped with epifluorescence. For body-length measurements, adult animals were measured 24 h after the final molt under ×100 magnification using Nomarski optics and an eyepiece micrometer. For pheromone hypersensitivity assays, animals were allowed to lay 50–100 eggs at room temperature on 6-cm plates with or without pheromone. Parents were then removed and the plates were incubated at 25°C. Dauer and non-dauer animals were counted 48 h later.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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References

Al-Khalili L, Chibalin AV, Yu M, Sjodin B, Nylen C, Zierath JR, Krook A (2004) MEF2 activation in differentiated primary human skeletal muscle cultures requires coordinated involvement of parallel pathways. *Am J Physiol Cell Physiol* **286:** C1410–C1416

Albert PS, Brown SJ, Riddle DL (1981) Sensory control of dauer larva formation in *Caenorhabditis elegans*. *J Comp Neurol* **198:** 435–451

Bandyopadhyay J, Lee J, Lee J, Lee JI, Yu JR, Jee C, Cho JH, Jung S, Lee MH, Zannoni S, Singson A, Kim do H, Koo HS, Ahnn J (2002) Calcineurin, a calcium/calmodulin-dependent protein phosphatase, is involved in movement, fertility, egg laying, and growth in *Caenorhabditis elegans*. *Mol Biol Cell* **13:** 3281–3293

Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* **77:** 71–94

Broday L, Kolotuev I, Didier C, Bhoumik A, Gupta BP, Sternberg PW, Podbilewicz B, Ronai Z (2004) The small ubiquitlin-like modifier (SUMO) is required for gonadal and uterine-vulval morphogenesis in *Caenorhabditis elegans*. *Genes Dev* **18:** 2380–2391

Cases I, de Lorenzo V (2005) Promoters in the environment: transcriptional regulation in its natural context. *Nat Rev Microbiol* **3:** 105–118

Chawla S, Vanhoutte P, Arnold FJ, Huang CL, Bading H (2003) Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J Neurochem* **85:** 151–159

Choi KY, Ji YJ, Jee C, Kim do H, Ahnn J (2002) Characterization of CeHDA-7, a class II histone deacetylase interacting with MEF-2 in *Caenorhabditis elegans*. *Biochem Biophys Res Commun* **293:** 1295–1300

Clark DA, Biron D, Sengupta P, Samuel ADT (2006) The AFD sensory neurons encode multiple functions underlying thermotactic behavior in *C. elegans*. *J Neurosci* **26:** 7444–7451

Colosimo ME, Brown A, Mukhopadhyay S, Gabel C, Lanjuin AE, Samuel AD, Sengupta P (2004) Identification of thermosensory and olfactory neuron-specific genes via expression profiling of single neuron types. *Curr Biol* **14:** 2245–2251

Dichoso D, Brodigan T, Chwoe KY, Lee JS, Llacer R, Park M, Corsi AK, Kostas SA, Fire A, Ahnn J, Krause M (2000) The MADS-Box factor CeMEF2 is not essential for *Caenorhabditis elegans* myogenesis and development. *Dev Biol* **223:** 431–440

Dwyer ND, Troemel ER, Sengupta P, Bargmann CI (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. *Cell* **93:** 455–466

Feldman JD, Vician L, Crispino M, Hoe W, Baudry M, Herschman HR (2000) The salt-inducible kinase, SIK, is induced by depolarization in brain. *J Neurochem* **74:** 2227–2238

Flavell SW, Cowan CW, Kim TK, Greer PL, Lin Y, Paradis S, Griffith EC, Hu LS, Chen C, Greenberg ME (2006) Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science* **311:** 1008–1012

Fujiwara M, Sengupta P, McIntire S (2002) Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron* **36:** 1091–1102

Gaudilliere B, Shi Y, Bonni A (2002) RNA interference reveals a requirement for myocyte enhancer factor 2A in activity-dependent neuronal survival. *J Biol Chem* **277:** 46442–46446

Golden JW, Riddle DL (1984) The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev Biol* **102:** 368–378

Gong X, Tang X, Wiedmann M, Wang X, Peng J, Zheng D, Blair LA, Marshall J, Mao Z (2003) Cdk5-mediated inhibition of the protective effects of transcription factor MEF2 in neurotoxicity-induced apoptosis. *Neuron* **38:** 33–46

Gossett LA, Kelvin DJ, Sternberg EA, Olson EN (1989) A new myocyte-specific enhancer-binding factor that recognizes a conserved element associated with multiple muscle-specific genes. *Mol Cell Biol* **9:** 5022–5033

Gregoire S, Tremblay AM, Xiao L, Yang Q, Ma K, Nie J, Mao Z, Wu Z, Giguere V, Yang XJ (2006) Control of MEF2 transcriptional activity by coordinated phosphorylation and sumoylation. *J Biol Chem* **281:** 4423–4433

Heidenreich KA, Linseman DA (2004) Myocyte enhancer factor-2 transcription factors in neuronal differentiation and survival. *Mol Neurobiol* **29:** 155–166

Hobert O (2002) PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic *C. elegans*. *Biotechniques* **32:** 728–730

Holmes BF, Sparling DP, Olson AL, Winder WW, Dohm GL (2005) Regulation of muscle GLUT4 enhancer factor and myocyte enhancer factor 2 by AMP-activated protein kinase. *Am J Physiol Endocrinol Metab* **289:** E1071–E1076

Horike N, Takemori H, Katoh Y, Doi J, Min L, Asano T, Sun XJ, Yamamoto H, Kasayama S, Muraoka M, Nonaka Y, Okamoto M (2003) Adipose-specific expression, phosphorylation of Ser794 in insulin receptor substrate-1, and activation in diabetic animals of salt-inducible kinase-2. *J Biol Chem* **278:** 18440–18447

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab* **1:** 15–25

Kang J, Gocke CB, Yu H (2006) Phosphorylation-facilitated sumoylation of MEF2C negatively regulates its transcriptional activity. *BMC Biochem* **7:** 5

Kao HY, Verdel A, Tsai CC, Simon C, Juguilon H, Khochbin S (2001) Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. *J Biol Chem* **276:** 47496–47507

Kim MS, Lee KU (2005) Role of hypothalamic 5′-AMP-activated protein kinase in the regulation of food intake and energy homeostasis. *J Mol Med* **83:** 514–520

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G (1997) *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277:** 942–946

Koo SH, Flechner L, Qi L, Zhang X, Screaton RA, Jeffries S, Hedrick S, Xu W, Boussouar F, Brindle P, Takemori H, Montminy M (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* **437:** 1109–1111

Kuhara A, Inada H, Katsura I, Mori I (2002) Negative regulation and gain control of sensory neurons by the *C. elegans* calcineurin TAX-6. *Neuron* **33:** 751–763

Lanjuin A, Sengupta P (2002) Regulation of chemosensory receptor expression and sensory signaling by the KIN-29 Ser/Thr kinase. *Neuron* **33:** 369–381

Lanjuin A, Sengupta P (2004) Specification of chemosensory neuron subtype identities in *Caenorhabditis elegans*. *Curr Opin Neurobiol* **14:** 22–30

Lee RY, Lobel L, Hengartner M, Horvitz HR, Avery L (1997) Mutations in the alpha1 subunit of an L-type voltage-activated Ca^{2+} channel cause myotonia in *Caenorhabditis elegans*. *EMBO J* **16:** 6066–6076

Li M, Linseman DA, Allen MP, Meintzer MK, Wang X, Laessig T, Wierman ME, Heidenreich KA (2001) Myocyte enhancer factor 2A and 2D undergo phosphorylation and caspase-mediated degradation during apoptosis of rat cerebellar granule neurons. *J Neurosci* **21:** 6544–6552

Linseman DA, Bartley CM, Le SS, Laessig TA, Bouchard RJ, Meintzer MK, Li M, Heidenreich KA (2003) Inactivation of the myocyte enhancer factor-2 repressor histone deacetylase-5 by endogenous $Ca^{(2+)}$//calmodulin-dependent kinase II promotes depolarization-mediated cerebellar granule neuron survival. *J Biol Chem* **278:** 41472–41481

Liu Y, Randall WR, Schneider MF (2005) Activity-dependent and -independent nuclear fluxes of HDAC4 mediated by different kinases in adult skeletal muscle. *J Cell Biol* **168:** 887–897

Lizcano JM, Goransson O, Toth R, Deak M, Morrice NA, Boudeau J, Hawley SA, Udd L, Makela TP, Hardie DG, Alessi DR (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamiy, including MARK/PAR-1. *EMBO J* **23:** 833–843

Lu J, McKinsey TA, Nicol RL, Olson EN (2000a) Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proc Natl Acad Sci USA* **97:** 4070–4075

Lu J, McKinsey TA, Zhang CL, Olson EN (2000b) Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol Cell* **6:** 233–244

Maduzia LL, Roberts AF, Wang H, Lin X, Chin LJ, Zimmerman CM, Cohen S, Feng XH, Padgett R (2005) *C. elegans* serine-threonine kinase KIN-29 modulates TGFβ signaling and regulates body size formation. *BMC Dev Biol* **5:** 8

Mao Z, Bonni A, Xia F, Nadal-Vicens M, Greenberg ME (1999) Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science* **286:** 785–790

Mao Z, Wiedmann M (1999) Calcineurin enhances MEF2 DNA binding activity in calcium-dependent survival of cerebellar granule neurons. *J Biol Chem* **274:** 31102–31107

Markstein M, Zinzen R, Markstein P, Yee KP, Erives A, Stathopoulos A, Levine M (2004) A regulatory code for neurogenic gene expression in the *Drosophila* embryo. *Development* **131:** 2387–2394

McCarroll SA, Li H, Bargmann CI (2005) Identification of transcriptional regulatory elements in chemosensory receptor genes by probabilistic segmentation. *Curr Biol* **15:** 347–352

McGee SL, Hargreaves M (2006) Exercise and skeletal muscle glucose transporter 4 expression: molecular mechanisms. *Clin Exp Pharm Phys* **33:** 395–399

McKinsey TA, Zhang CL, Lu J, Olson EN (2000a) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408:** 106–111

McKinsey TA, Zhang CL, Olson EN (2000b) Activation of the myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. *Proc Natl Acad Sci USA* **97:** 14400–14405

McKinsey TA, Zhang CL, Olson EN (2002) MEF2: a calcium-dependent regulator of cell division, differentiation and death. *Trends Biochem Sci* **27:** 40–47

Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferre P, Birnbaum MJ, Stuck BJ, Kahn BB (2004) AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **428:** 569–574

Nolan KM, Sarafi-Reinach TR, Horne JG, Saffer AM, Sengupta P (2002) The DAF-7 TGF-beta signaling pathway regulates chemosensory receptor gene expression in *C. elegans*. *Genes Dev* **16:** 3061–3073

Ogura K, Shirakawa M, Barnes TM, Hekimi S, Ohshima Y (1997) The UNC-14 protein required for axonal elongation and guidance in *Caenorhabditis* elegans interacts with the serine/threonine kinase UNC-51. *Genes Dev* **11:** 1801–1811

Okamoto M, Takemori H, Katoh Y (2004) Salt-inducible kinase in steroidogenesis and adipogenesis. *Trends Endocrinol Metab* **15:** 21–26

Peckol EL, Troemel ER, Bargmann CI (2001) Sensory experience and sensory activity regulate chemosensory receptor gene expression in *C. elegans*. *Proc Natl Acad Sci USA* **98:** 11032–11038

Pollock R, Treisman R (1991) Human SRF-related proteins: DNA-binding properties and potential regulatory targets. *Genes Dev* **5:** 2327–2341

Reiner DJ, Newton EM, Tian H, Thomas JH (1999) Diverse behavioural defects caused by mutations in *Caenorhabditis elegans unc-43* CaM kinase II. *Nature* **402:** 199–203

Riquelme C, Barthel KK, Liu X (2006) SUMO-1 modification of MEF2A regulates its transcriptional activity. *J Cell Mol Med* **10:** 132–144

Sagasti A, Hobert O, Troemel ER, Ruvkun G, Bargmann CI (1999) Alternative olfactory neuron fates are specified by the LIM homeobox gene *lim-4*. *Genes Dev* **13:** 1794–1806

Schafer WR, Sanchez BM, Kenyon CJ (1996) Genes affecting sensitivity to serotonin in *Caenorhabditis elegans*. *Genetics* **143:** 1219–1230

Screaton RA, Conkright MD, Katoh Y, Best JL, Canettieri G, Jeffries S, Guzman E, Niessen S, Yates III JR, Takemori H, Okamoto M, Montminy M (2004) The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. *Cell* **119:** 61–74

Shalizi A, Gaudilliere B, Yuan Z, Stegmuller J, Shirogane T, Ge Q, Tan Y, Schulman B, Harper JW, Bonni A (2006) A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science* **311:** 1012–1017

Simmer F, Moorman C, Van Der Linden AM, Kuijk E, Van Den Berghe PV, Kamath R, Fraser AG, Ahringer J, Plasterk RH (2003) Genome-wide RNAi of *C. elegans* using the hypersensitive *rrf-3* strain reveals novel gene functions. *PLoS Biol* **1:** 77–84

Sze JY, Victor M, Loer C, Shi Y, Ruvkun G (2000) Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* **403:** 560–564

Tobin D, Madsen D, Kahn-Kirby A, Peckol E, Moulder G, Barstead R, Maricq A, Bargmann CI (2002) Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. *Neuron* **35:** 307–318

Troemel ER, Kimmel BE, Bargmann CI (1997) Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* **91:** 161–169

Troemel ER, Sagasti A, Bargmann CI (1999) Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. *Cell* **99:** 387–398

Wang Z, Takemori H, Halder SK, Nonaka Y, Okamoto M (1999) Cloning of a novel kinase (SIK) of the SNF1/AMPK family from high salt diet-treated rat adrenal. *FEBS Lett* **453:** 135–139

Wasserman WW, Fickett JW (1998) Identification of regulatory regions which confer muscle-specific gene expression. *J Mol Biol* **278:** 167–181

White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Phil Trans R Soc London B* **314:** 1–340

Wu H, Naya FJ, McKinsey TA, Mercer B, Shelton JM, Chin ER, Simard AR, Michel RN, Bassel-Duby R, Olson EN, Williams RS (2000) MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *EMBO J* **19:** 1963–1973

Wu H, Rothermel B, Kanatous S, Rosenberg P, Naya FJ, Shelton JM, Hutcheson KA, DiMaio JM, Olson EN, Bassel-Duby R, Williams RS (2001) Activation of MEF2 by muscle activity is mediated through a calcineurin-dependent pathway. *EMBO J* **20:** 6414–6423

Wyrick JJ, Young RA (2002) Deciphering gene expression regulatory networks. *Curr Opin Genet Dev* **12:** 130–136

Yang XJ, Gregoire S (2005) Class II histone deacetylases: from sequence to function, regulation, and clinical implication. *Mol Cell Biol* **25:** 2873–2884

Yuh CH, Davidson EH (1996) Modular *cis*-regulatory organization of *Endo16*, a gut-specific gene of the sea urchin embryo. *Development* **122:** 1069–1082

