indicated. The *mrt-2* arrays were crossed into the various genetic backgrounds via Bristol N2.

To determine the sensitivity of the *mrt-2* transgenic strains to ionizing radiation, L4 larvae were irradiated with 60 Gy, using a ^{137}Cs source. After 48 h, single animals were selected; their progeny was scored for survival 24 h later.

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Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2

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Membrane-associated guanylate kinases (MAGUKs) contain multiple protein-binding domains that allow them to assemble specific multiprotein complexes in particular regions of the cell[1,2]. CASK/LIN-2, a MAGUK required for EGF receptor localization and signalling in *Caenorhabditis elegans*, contains a calmodulin-dependent protein kinase-like domain followed by PDZ, SH3 and guanylate kinase-like domains[3–5]. In adult rat brain, CASK is concentrated at neuronal synapses and binds to the cell-surface proteins neurexin and syndecan[6–8] and the cytoplasmic proteins Mint/LIN-10 and Veli/LIN-7 (refs 4, 9, 10). Here we report that, through its guanylate kinase domain, CASK interacts with Tbr-1, a T-box transcription factor that is involved in forebrain development[11,12]. CASK enters the nucleus and binds to a specific DNA sequence (the T-element) in a complex with Tbr-1. CASK acts as a coactivator of Tbr-1 to induce transcription of T-element containing genes, including reelin, a gene that is essential for cerebrocortical development. Our findings show that a MAGUK which is usually associated with cell junctions has a transcription regulation function.

To identify binding partners for the guanylate kinase (GK) domain of CASK, we carried out a yeast two-hybrid screen of brain complementary DNA libraries, from which Tbr-1 was isolated (Fig. 1a). Tbr-1 bound to the GK domain of CASK but not the GK domain of PSD-95. By deletion analysis, the carboxy-terminal region of Tbr-1 (residues 342–681) was found to be necessary and sufficient for association with the GK domain of CASK (Fig. 1a). A biochemical association of full-length CASK and Tbr-1 was confirmed in mammalian cells. When co-expressed in COS-7 cells, Tbr-1 and CASK were readily co-precipitated by antibodies directed against either individual protein (Fig. 1b). Chapsyn-110, a member of the PSD-95 subfamily of MAGUKs, was not co-precipitated with Tbr-1 (Fig. 1b). These biochemical data support the yeast two-hybrid results (Fig. 1a), confirming a specific interaction of Tbr-1 and CASK.

If CASK interacts physiologically with Tbr-1, it might redistribute into the nucleus in a Tbr-1-dependent fashion. After transfection in COS cells, Tbr-1 immunoreactivity was concentrated in nuclei (Fig. 2a). CASK, when expressed by itself, was cytoplasmic and relatively excluded from nuclei of COS-7 cells (Fig. 2b). Upon co-expression with Tbr-1, however, CASK redistributed into nuclei where it co-localized with Tbr-1 (Fig. 2e). This nuclear translocation is specific for CASK, because Tbr-1 did not cause the nuclear localization of other MAGUK proteins, for example, chapsyn-110 (Fig. 2c, h) and PSD-95 (Fig. 2d, i). A deletion mutant of CASK lacking the GK domain remained predominantly cytoplasmic even in the presence of nuclear Tbr-1 (Fig. 2f). Moreover, a mutant of Tbr-1 lacking the CASK-binding C-terminal region was unable to redistribute CASK into the nucleus, even though this Tbr-1 mutant itself targeted to nuclei (Fig. 2g). The nuclear localization of CASK

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therefore depends on the interaction between the C-terminal region of Tbr-1 and the GK domain of CASK.

When co-transfected with haemagglutin in A (HA)-tagged Tbr-1 into cultured hippocampal neurons, CASK similarly accumulated in the nucleus with Tbr-1 (Fig. 2k). Transfection of HA–Tbr-1 alone caused endogenous CASK to partition into neuronal nuclei (Fig. 2j). Thus Tbr-1-dependent nuclear translocation of CASK occurs in neurons as well as heterologous cells.

We considered whether the Tbr-1-dependent nuclear localization of CASK could be influenced by binding partners of CASK that are usually localized to the cell surface, such as the syndecan family of transmembrane heparan sulphate proteoglycans[7,13]. When syndecan-3 was co-expressed with Tbr-1 and CASK in COS cells, CASK no longer accumulated specifically in the nucleus but instead co-localized with syndecan-3 in a perinuclear endoplasmic reticulum (ER)-like pattern in the cytoplasm (compare Fig. 2l, middle, with 2e, left), even though Tbr-1 was concentrated in the nucleus of the same cell (Fig. 2l, right). These data suggest that the subcellular distribution of CASK may be regulated by the balance of its various binding partners in different subcellular compartments.

Does the Tbr-1–CASK complex bind to a specific DNA regulatory element? As the T-box of Tbr-1 is closely related to the T-box of Brachyury, we first examined whether Tbr-1 can recognize the 'T-element', the DNA target sequence for Brachyury[14]. In gel-mobility shift assays, nuclear extracts purified from COS cells expressing Tbr-1 caused a retardation of the T-element oligonucleotide probe (Fig. 3a, lane 3, band I). Peptide antibodies raised against either the carboxy or amino terminus of Tbr-1 (TBR-C and TBR-N) caused a supershift of this band, indicating that the retarded complex contains Tbr-1 (Fig. 3a, lanes 4 and 5, band II). Competition controls with unlabelled oligonucleotides confirmed the specificity of T-element binding of Tbr-1 (data not shown).

We tested whether CASK can associate with Tbr-1 in a DNA-binding complex in two ways. First, the GK domain of CASK was added as a purified gluthathione *S*-transferase (GST) fusion protein to Tbr-1 nuclear extracts in the presence of the T-element probe. Addition of GK domain of CASK caused a further retardation of the Tbr-1–DNA complex in a dose-dependent manner (Fig. 3b, lanes 12–14, arrow), whereas GST alone had no effect (lane 11). TBR-N antibodies supershifted this GST–CASK–Tbr-1–T-element complex (Fig. 3b, lane 15, asterisk), confirming that this complex contains Tbr-1. Second, nuclear extracts were prepared from COS cells co-transfected with CASK and Tbr-1 (Fig. 3c). These CASK–Tbr-1 nuclear extracts shifted the T-element oligo to a major band similarly to Tbr-1 alone (lane 20, band 'I'), suggesting that Tbr-1 is in excess in these nuclear extracts or that the CASK–Tbr-1–DNA complex migrates similarly to the Tbr-1–DNA complex. This complex could be supershifted with TBR-N or TBR-C antibodies, as expected (lanes 21, 22, band II). Significantly, antibodies to CASK also caused a similar, albeit weaker, supershift in Tbr-1–CASK nuclear extract (lane 23), which was not seen in nuclear extracts expressing Tbr-1 alone (lane 19), or Tbr-1 and CASKΔGK (lane 28). Thus, CASK is present in a T-element-binding complex, and formation of this complex requires the GK domain of CASK. Notably, when antibodies to both CASK and Tbr-1 are added to CASK–Tbr-1 nuclear extracts, a new 'supersupershifted' T-element complex was formed that barely entered the gel (lanes 24–25, band III). This slow-migrating complex, which presumably contained T-element, Tbr-1, and CASK plus antibodies to Tbr-1 and CASK, was not seen with the Tbr-1–CASKΔGK nuclear extract (Fig. 3c, lanes 26–27), indicating that the GK domain is required for CASK to associate with Tbr-1 and the T-element DNA. Collectively, the gel-retardation data show that CASK forms a GK domain-dependent complex with Tbr-1 that binds to the T-element DNA sequence.

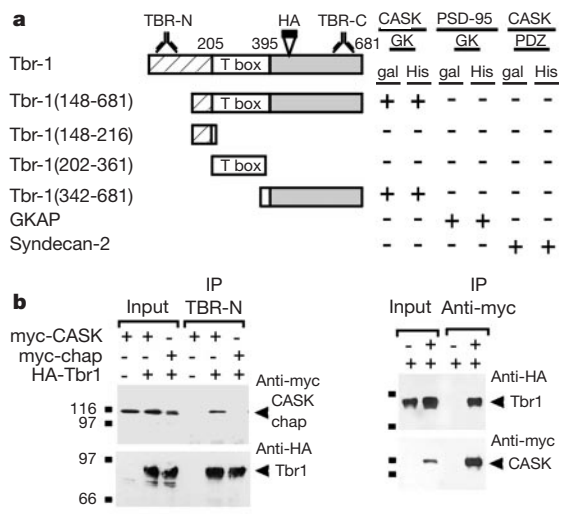

Figure 1 Specific interaction of CASK and Tbr-1. **a**, Yeast two-hybrid analysis of CASK/Tbr-1 interaction. Full-length Tbr-1 is shown at the top. Tbr-1 cDNA isolated from the yeast two-hybrid screen (residues 148–681) and other deletion constructs are aligned beneath. Interaction with the GK domain (residues 684–909) of CASK, the GK domain of PSD-95 and the PDZ domain of CASK are summarized, based on induction of reporter genes β-galactosidase (gal) and HIS3 (His)[21]. GKAP and syndecan-2 were used as positive controls for interaction with the GK domain of PSD-95 and PDZ domain of CASK, respectively[7,22]. **b**, Co-immunoprecipitation of CASK and Tbr-1 from COS-7 cells. Cells were co-transfected with various combinations of Myc-tagged CASK, HA-tagged Tbr-1, and Myc-tagged chapsyn-110, and extracts were immunoprecipitated (IP) with TBR-N or anti-Myc antibodies, as indicated. Immunoprecipitates were immunoblotted using Myc and HA antibodies, as indicated. CASK and chapsyn-110 (chap) co-migrate on SDS–PAGE. Input lanes contain 5% of extract used for immunoprecipitation.

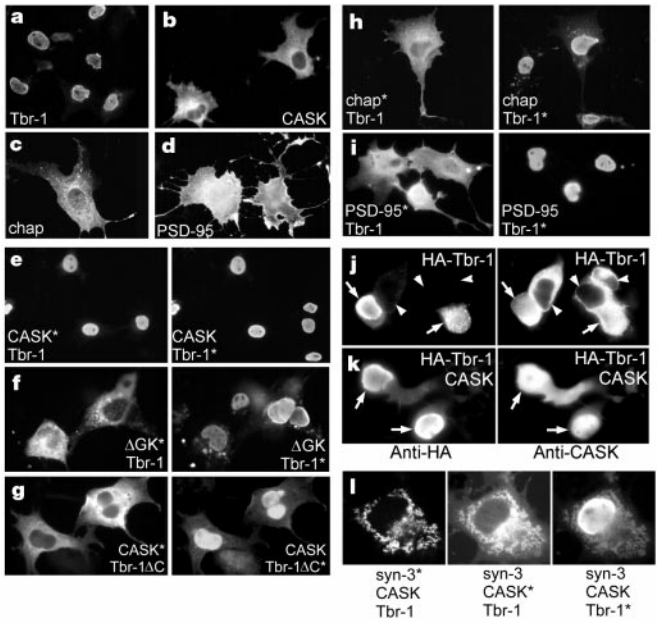

Figure 2 Tbr-1-dependent nuclear translocation of CASK. COS-7 cells were transfected with single cDNAs (**a**–**d**) or co-transfected with two or three different cDNAs (**e**–**i**, **l**), as identified in each panel. The subcellular distribution of transfected proteins was visualized by indirect immunofluorescence. For co-transfected cells, we used double-labelling (**e**–**i**) and triple labelling (**l**). Each group of images (in **e**–**l**) represents the same field viewed with FITC, Cy3 or Cy5 filters; the protein that is specifically visualized is marked by an asterisk. ΔGK, CASK mutant deleted for the GK domain (residues 708–909); Tbr-1ΔC, a deletion mutant of Tbr-1 lacking the C-terminal region (residues 401–681); chap, chapsyn-110; syn-3, syndecan-3. Similar results were obtained in HEK293 cells (not shown). **j**, **k**, double staining for HA–Tbr-1 and CASK in cultured hippocampal neurons transfected with HA–Tbr-1 alone (**j**) or both HA–Tbr-1 and CASK (**k**). Left images in **j** and **k** are stained for HA–Tbr-1; right images for CASK. Arrows indicate nuclei of transfected neurons; arrowheads point to nuclei of untransfected cells.

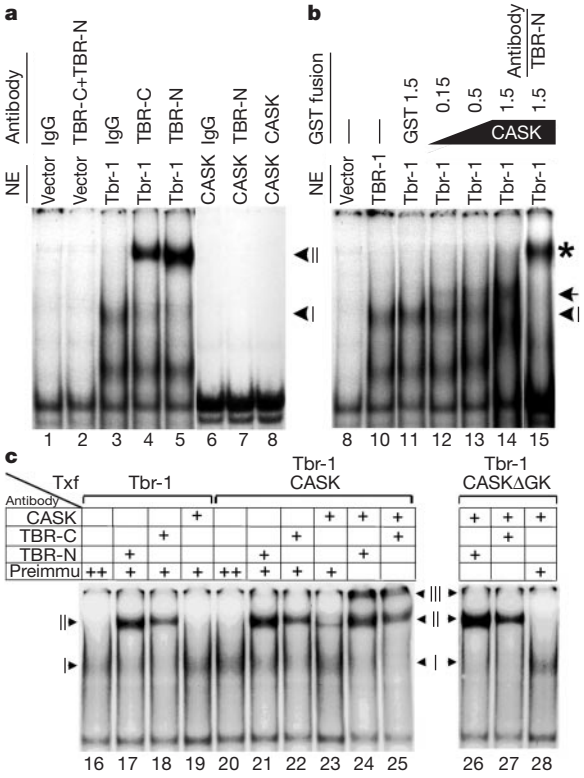

Figure 3 Tbr-1–CASK complex binds to T-element DNA. **a**, Tbr-1 binds the T-element in gel-mobility shift assays. Labelled T-element oligonucleotide was mixed with nuclear extract (NE) from COS-7 cells transfected with Tbr-1, CASK or vector control. In addition, protein-A-purified TBR-N, TBR-C and CASK antibodies, or control non-immune antibodies (IgG), were added to supershift Tbr-1-containing DNA complexes. **b**, GK domain of CASK supershifts the Tbr-1–DNA complex. Increasing amounts (0.15, 0.5 and 1.5 μg) of purified GST-fusion protein containing the GK domain of CASK were added to Tbr-1 nuclear extract and the mixtures were analysed by T-element gel-shift assay. GST alone (1.5 μg) was used as a negative control. **c**, Ternary complex of full-length CASK, Tbr-1 and T-element. Nuclear extracts from COS-7 cells transfected (Txf) with Tbr-1 alone or in combination with CASK or CASKΔGK, as indicated, were used in T-element gel-shift assays. Each reaction contained anti-CASK, anti-Tbr-1 or pre-immune IgG, as indicated. The total amount of antibody was equalized by addition of pre-immune IgG. Retarded protein–DNA complexes are labelled I, II and III.

Tbr-1 is more highly expressed in embryonic brain[11] (Fig. 4a), therefore the interaction between Tbr-1 and CASK may be particularly important in developing brain. Expression of CASK is roughly constant from embryonic day E17 to postnatal day P42. A significant fraction of CASK is present in nuclei purified from E17 and P5 cerebral cortex (Fig. 4a). Roughly 10–20% of CASK was present in nuclei at E17, and this percentage declined with development; at P42, nuclear CASK was barely detectable by immunoblotting. Several cytoplasmic proteins (chapsyn-110, β-tubulin, dynamin, MAP2c) were not detected in the purified nuclear fractions; thus, the CASK signal in E17 and P5 nuclei is unlikely to represent cytoplasmic contamination.

To examine whether Tbr-1 and CASK coexist in the same nuclei of prenatal neocortex, we carried out double-label immunofluorescence confocal microscopy on E19 brain sections. As expected, Tbr-1 was localized in the nuclei of neurons (Fig. 4c, right). CASK was diffusely distributed, mainly in a cytoplasmic pattern. However, there was weak but significant immunoreactivity for CASK in nuclei (Fig. 4c, left), resulting in a relatively 'filled-in' appearance of the nuclei compared with MAP2 staining at E19 (Fig. 4d, left) or CASK staining at P42 (Fig. 4e, left), both of which were excluded from nuclei. We used quantitative immunofluorescence to estimate the fraction of CASK in nuclei (Fig. 4f). About 20% of CASK immunofluorescence was localized to nuclei in E19 rat brain, consistent with immunoblotting data (Fig. 4a). In contrast, nuclear immunoreactivity of MAP2 in E19 rat brain represented only ~3% of the total, and nuclear CASK in P42 brain was undetectable (Fig. 4f).

We could co-immunoprecipitate Tbr-1 (but not PSD-95) from embryonic cortex using CASK antibodies (Fig. 4b, left), and vice versa (Fig. 4b, right), providing more evidence for the association of CASK and Tbr-1 *in vivo*. We were unable to co-immunoprecipitate CASK and Tbr-1 from P42 brain when expression of Tbr-1 was low.

There is a change in the subcellular distribution of Tbr-1 and CASK during cortical development (Fig. 4c, e). In P42 cortex, CASK is found in a punctate pattern in the neuropil, with additional cytoplasmic staining in neuronal cell bodies (Fig. 4e, left). At this stage, Tbr-1 is still present in the nucleus of neurons, but no longer restricted to nuclei as it was at E19. Instead, Tbr-1 distribution extends into the cytoplasm of P42 neurons (Fig. 4e, right), overlapping considerably with CASK outside of the nucleus.

What is the functional consequence of CASK binding on the putative transcriptional activity of Tbr-1? First, we tested whether

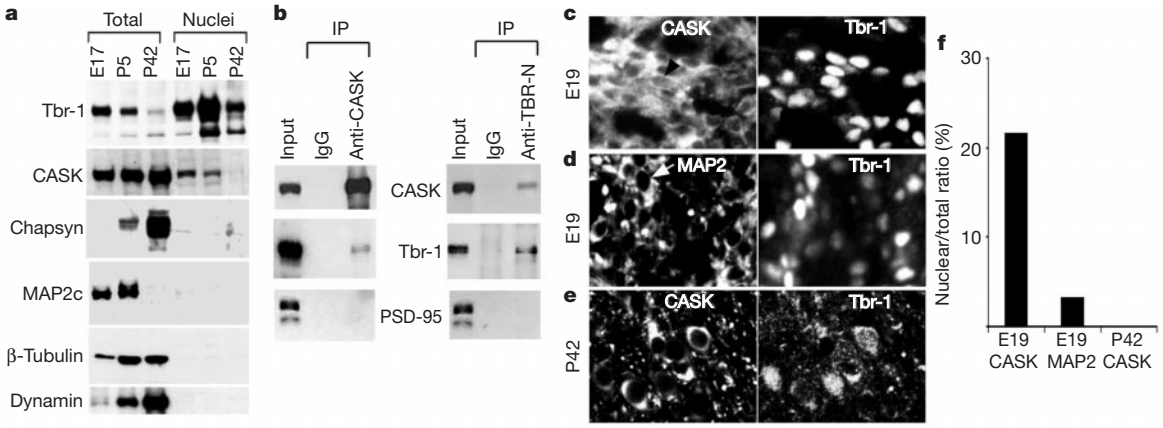

Figure 4 CASK is found with Tbr-1 in nuclei of neurons in embryonic rat cerebral cortex. **a**, Total homogenate and purified nuclei from cortex at different ages (E17, P5, P42) were immunoblotted for various proteins, as indicated. Ten micrograms of protein from each fraction, except P5 nuclei, was applied to SDS–PAGE. Because of an error in quantitation of protein, the P5 nuclei lane actually contained ~25 μg of protein. **b**, Co-immunoprecipitation of CASK and Tbr-1 from embryonic rat cortex. Triton-X-100 extract of E20 cortex was immunoprecipitated with TBR-N or CASK antibodies, or non-immune IgG, as indicated. The precipitates were immunoblotted for CASK, Tbr-1 and PSD-95. Input lanes contain 15% of extract used for immunoprecipitation. **c**–**e**, Double-label fluorescent immunostaining for CASK and Tbr-1, or MAP2 and Tbr-1, as indicated. **c**, **d**, E19 olfactory bulb (similar results were seen in cerebral cortex); **e**, P42, layer 5 of cerebral cortex. Arrowhead in **c** indicates an example of a nucleus with CASK staining. Arrow in **d** illustrates the absence of MAP2 in nuclei in a parallel section. **f**, Quantitative analysis of nuclear/total ratio of CASK and MAP2 immunofluorescence determined from confocal images at E19 and P42.

Tbr-1 affects the expression of a luciferase reporter gene placed under the control of an upstream T-element (T-luc) in heterologous cells. Consistent with earlier studies[14], Brachyury strongly stimulated T-luc expression (~20-fold, Fig. 5a). In comparison, Tbr-1 induction of T-luc expression was weak (~two–threefold relative to vector control). Co-expression of CASK, however, enhanced the transcription stimulation by Tbr-1 to ~10-fold (Fig. 5a). A CASK mutant with deleted GK domain (CASKΔGK) was unable to stimulate Tbr-1 activation of T-luc, and wild-type CASK had no effect on the transcription activity of Brachyury. In addition, CASK itself had no significant effect on T-luciferase expression (Fig. 5a). We conclude that CASK specifically stimulates transcriptional activity of Tbr-1 by GK-dependent binding to Tbr-1.

A fusion protein of the GAL-4 DNA-binding domain and the C-terminal region of Tbr-1 (GAL-4–Tbr-1C) had no effect on the GAL-4-luciferase reporter gene (Fig. 5b), suggesting that the CASK-binding domain of Tbr-1 itself has no transcriptional activity. However, co-expression of CASK with GAL-4–Tbr-1C greatly stimulated GAL4-luc expression (Fig. 5b), confirming that CASK can act as a 'coactivator' of Tbr-1. CASK mutants individually lacking calmodulin-dependent protein kinase (CaMK), Veli/LIN-7-binding, PDZ or SH3 domains were still able to coactivate GAL4-luc transcription in the presence of GAL-4–Tbr-1C, but deleting the GK domain abolished this activity (Fig. 5b). Moreover, the GK domain alone was as effective as full-length CASK (Fig. 5b); thus, the GK domain of CASK is necessary and sufficient for the coactivator activity of CASK on Tbr-1.

We also tested the transcriptional effects of Tbr-1 and CASK in neurons. Overexpression of Tbr-1 in cultured neurons stimulated expression of the co-transfected T-luc reporter gene (Fig. 6a). Notably, in contrast to the data from COS cells, transfection of CASK alone was able to induce T-luc expression in cultured neurons (>10-fold; Fig. 6a). This result can be explained by the fact that Tbr-1 is endogenously expressed in these neurons (data not shown). Co-transfection of both CASK and Tbr-1 further enhanced T-luc activity (Fig. 6a). The stimulatory effect of CASK on T-element-directed transcription therefore occurs in neurons as well as heterologous cells.

Tbr-1 expression is highly restricted to cerebral cortex, particularly at prenatal stages[11], and is undetectable in lung, heart, liver and kidney of E20 rat embryos (data not shown), suggesting that Tbr-1 may have a role in cerebral cortex development. Consistent with this idea, Tbr-1 knockout mice show a developmental phenotype similar to Reeler mice, which is characterized by abnormal lamination of cerebral cortex[12]. We noticed that the 5′ upstream region of the reelin gene (which is mutated in Reeler mice) contains two half-palindromes of the T-element, and we proposed that reelin gene transcription may be controlled by Tbr-1–CASK. To test this idea, we determined in transfected neurons the activity of Tbr-1 and CASK on a luciferase reporter gene under the control of the reelin 5′ upstream region (Reelin-luc). Tbr-1 itself stimulated Reelin-luc expression only modestly (~twofold relative to control vector; Fig. 6b), perhaps because Tbr-1 binds weakly to half-palindromes of the T-element. Overexpression of CASK by itself greatly induced expression of Reelin-luc in neurons (~16 fold; Fig. 6b). Moreover, when co-transfected with both CASK and Tbr-1, expression of Reelin-luc was further enhanced (~28 fold; Fig. 6b). In addition to confirming the transcriptional stimulatory activity of CASK, these results indicate that the Tbr-1–CASK complex can positively regulate a natural gene promoter in neurons and suggest that reelin is an endogenous target gene of Tbr-1–CASK.

We have reported the first example, to our knowledge, of a MAGUK translocating into the nucleus, interacting with a defined transcription factor and regulating transcription. Like other MAGUKs, CASK has been mainly characterized as a membrane-associated scaffold protein, involved in the assembly of specific protein complexes at sites of cell contact. By also regulating transcription, CASK invites analogies with β-catenin, a cadherin-binding protein that is associated with adherens junctions which can also function as a nuclear mediator by binding to Tcf/LEF

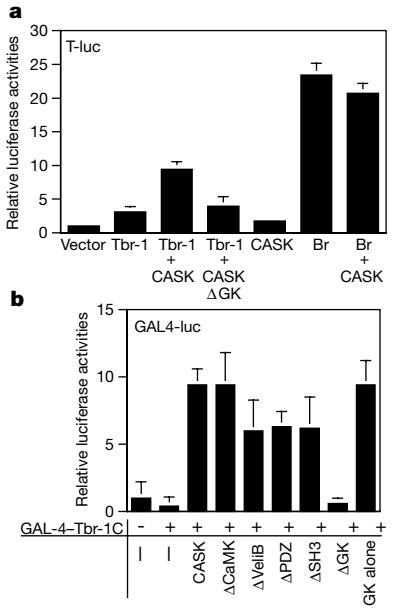

Figure 5 CASK stimulates transcriptional activity of Tbr-1 in COS-7 cells. **a**, Luciferase reporter gene under the control of T-element (T-luc) was co-transfected with Tbr-1, CASK, CASKΔGK, Brachyury (Br) or vector control, as indicated. **b**, GK domain of CASK is necessary and sufficient for coactivation effect of CASK. GAL-4-luciferase reporter gene (GAL-4-luc) was co-transfected with control vector, GAL-4-Tbr-1C, plus various CASK constructs, as indicated. Δ indicates the specific single-domain deletions of CASK. CASK, full-length CASK; GK alone, CASK GK domain alone. Luciferase activities are normalized to vector control. Error bars show standard deviations ($n = 6$).

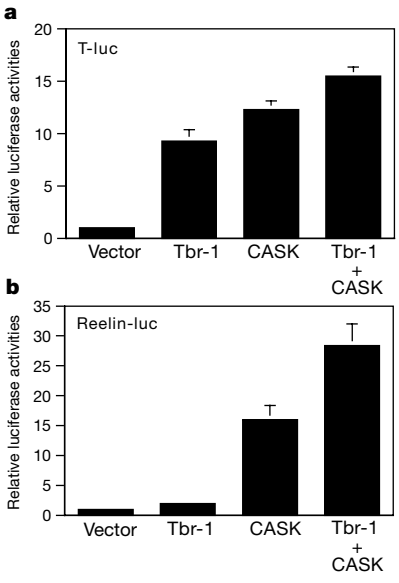

Figure 6 Tbr-1/CASK complex activates gene expression in cultured neurons. **a**, CASK–Tbr-1 stimulates T-luc expression. Hippocampal neurons (3DIV) were co-transfected with T-luc reporter and Tbr-1 and/or CASK, as indicated. **b**, CASK–Tbr-1 complex activates expression of luciferase reporter gene under the control of Reelin gene upstream region (Reelin-luc). Reelin-luc was co-transfected with Tbr-1 and/or CASK into cultured hippocampal neurons, as indicated. Error bars show standard deviations ($n = 3$).

transcription factors[15,16]. Another MAGUK (ZO-1) has been shown to shift from the tight junction to the nucleus in a cell contact-regulated manner, but the mechanism of this redistribution is unknown[17].

Although we detected nuclear CASK only in embryonic neurons, it remains possible that CASK translocates from cell junction to nucleus in mature neurons. Such a translocation may be highly regulated and/or of small magnitude, thus preventing the detection of CASK in the nuclei of mature neurons. In a heterologous system, we have found that a transmembrane ligand of CASK (syndecan-3) can shift the balance of CASK distribution between nucleus and cytoplasm (Fig. 2). Studying the dynamic regulation of CASK interactions with neurexins and syndecans may therefore be key to understanding the potential involvement of CASK in cell surface-to-nucleus signalling. □

Methods

Plasmid constructs

Tbr-1 coding sequence was RT-PCR amplified from mouse brain cDNA and subcloned into eukaryotic expression vector GW1-CMV (British Biotechnology). The HA epitope was inserted into a unique *Asc*I site (between residues 479–480) of Tbr-1. To construct T-luc, two repeats of T element (AATTTCACACCTAGGTGTGAAATT) were subcloned into reporter plasmid ΔERSV90-Luc, which contains the basal promoter of RSV fused to luciferase. Reelin-luc contains ~1 kb of 5′ upstream region of the reelin gene (GenBank accession number AC002067, nucleotides 3,700–4,620). GAL-4–Tbr-1C contains residues 342–681 of Tbr-1.

Transfection, immunoprecipitation, immunohistochemistry, luciferase assay

Transfection (using Lipofectamine), immunocytochemistry and immunoprecipitation of COS-7 cells were done as described[18]. For immunohistochemistry, rat brain sections were processed by indirect immunofluorescence as described[7], with the following modifications. E19 rat brains were fixed with 4% formaldehyde in PBS at 4 °C overnight, and cryoprotected with 20% sucrose in PBS. Frozen sections (25-μm) were collected on slides, air dried for at least 30 min, and permeabilized with methanol at −20 °C for 20 min. Results were viewed with an Axioskop microscope or a Bio-Rad MRC-1000 confocal microscope. For luciferase assays, COS-7 cells and cultured hippocampal neurons were harvested 18–20 h after transfection. Luciferase activity was measured in equal amounts of cell lysate protein using the Luciferase assay system (Promega).

Subcellular fractionaction from rat brain

Purification of nuclei from rat cerebral cortex was done as described[19].

DNA-binding gel-mobility shift assay

To prepare nuclear extracts, transfected COS-7 cells were harvested, quickly washed with hypotonic buffer (20 mM HEPES pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT), and resuspended in a volume of hypotonic buffer about fives times the packed cell volume. Cells were swollen at 4 °C for 10 min and broken by dounce homogenization with a type B pestle. Nuclei were collected and resuspended in buffer B (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 0.5 mM DTT, 20% glycerol) containing 0.3 M KCl. The nuclei were further extracted with gentle mixing at 4 °C for 30 min, after which nuclear debris was removed by centrifugation in a microcentrifuge at 14,000*g* for 10 min. Nuclear extracts were diluted by addition of two volumes of buffer B (reducing the concentration of KCl to 0.1 M) before use. Gel mobility shift assays were done as described[20]. The T-element oligonucleotides were annealed and labelled by T4 polynucleotide kinase with [γ-^{32}P]ATP.

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Phosphorylation of CPE binding factor by Eg2 regulates translation of c-*mos* mRNA

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Full-grown *Xenopus* oocytes arrest at the G2/M border of meiosis I. Progesterone breaks this arrest, leading to the resumption of the meiotic cell cycles and maturation of the oocyte into a fertilizable egg. In these oocytes, progesterone interacts with an unidentified surface-associated receptor, which induces a non-transcriptional signalling pathway that stimulates the translation of dormant c-*mos* messenger RNA. Mos, a mitogen-activated protein (MAP) kinase kinase kinase, indirectly activates MAP kinase, which in turn leads to oocyte maturation. The translational recruitment of c-*mos* and several other mRNAs is regulated by cytoplasmic polyadenylation, a process that requires two 3′ untranslated regions, the cytoplasmic polyadenylation element (CPE) and the polyadenylation hexanucleotide AAUAAA[1–4]. Although the signalling events that trigger c-*mos* mRNA polyadenylation and translation are unclear, they probably involve the activation of

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assembled with various bilins as described[15]. Spectra were obtained after saturating red (660 nm) and far-red (740 nm) irradiations. *In vitro* kinase assays for the active *Br*BphPs were performed as described[5].

Construction of *BrbphP* and *ppsR* mutant strains

To create *Brbph* and *ppsR* null mutants, the *lacZ-kan*^{r} cassette[19] was inserted respectively in the *Xho*I site of *BrbphP* and the *Bgl*II site of *ppsR*.The constructions were introduced in the pJQ200 suicide vector[22] and delivered by conjugation into the ORS278 strain as previously described[8]. Double recombinants were selected on sucrose and confirmed by PCR.

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Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to A.V. (e-mail: avermeglio@cea.fr). GenBank accession codes for the (bacterio)phytochrome sequences are AF182374 (*Bradyrhizobium* ORS278), AB00139 (*Synechocystis* PCC6803), AAF12261 (*D. radiodurans*), AF064527 (*R. centenum*), X17342 (*Arabidopsis thaliana*). The genomic organization of *Rps. palustris* was deduced from the genome database at http://spider.jgi-psf.org/JGI-microbial/html/.

corrigendum

Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2

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Nature **404**, 298–302 (2000).

In this Letter, we numbered some nucleotides for the upstream region of the *reelin* gene incorrectly. The Reelin-luc construct contains an upstream region of the *reelin* gene corresponding to nucleotides 157700–158620 of human BAC clone AC002067, instead of nucleotides 3700–4620. This does not affect any of the results or conclusions of the paper. We thank A. M. Goffinet, D. Grayson, K. Mendra and T. Curran for alerting us to this mistake. □

erratum

Origins and estimates of uncertainty in predictions of twenty-first century temperature rise

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Nature **416**, 723–726 (2002).

On page 725 of this Letter, the words 'predicts[13] fThur lglk al seasitevier' were corrupted. They should read 'predicts[13]. This lack of sensitivity'. □