tics also relate to caudate nucleus function adds to the evidence that TS and obsessive-compulsive disorder are overlapping neurobehavioral conditions.

The caudate nucleus is a key node in certain behavior-linked neural circuits, which are distinct from putamenal motor circuits (*18*). Our study may aid in understanding the curious motor behaviors of TS because it links these behaviors to associative, nonmotor striatal circuits and distinguishes them, as long suspected, from traditional hyperkinetic movement disorders that are linked to motor striatal circuits. Although there is insufficient basic neuroscience data to explain precisely how variation in D2 receptor availability in the head of the caudate nucleus accounts for the variable expression of TS, we speculate that involvement of the caudate nucleus may be related to the "compulsive" component of tics, a unique feature that helps to distinguish tics from other hyperkinetic movements. Dopaminergic dysfunction in the caudate nucleus may well be the common link between the ideational and motor components of TS.

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9. Twin pairs were solicited through the national newsletter of the Tourette Syndrome Association. Of nearly 100 responding twin pairs, 5 pairs fulfilled all selection criteria (adult, monozygotic, neuroleptic-free, and discordant for symptom severity). Complete matching of 19 red cell antigens (National Reference Laboratory for Blood Serology, American Red Cross, Rockville, MD) confirmed monozygosity with a probability of >97% [F. Vogel and A. G. Motulsky, *Human Genetics* (Springer-Verlag, New York, 1986) pp. 578–585]. Five participants were neuroleptic-naïve, three were neuroleptic-free for 3 years or more, and two discontinued low dosages of pimozide (1 mg/day) and haloperidol (1 mg/day), respectively, 6 weeks before the study. Diagnosis of TS followed published guidelines [S. Fahn *et al.*, *Arch. Neurol.* **50**, 1013 (1993)]. Patients were rated for symptom severity with an aggregate clinical score calculated as the sum of the following complementary scales: Shapiro Symptom Check List, Yale Global Tic Severity Rating Scale, and Yale Tic Count (performed immediately before SPECT scanning). Written informed consent was obtained from all participants under a protocol approved by the Institutional Review Board of the National Institute of Mental Health.
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11. [^{123}I]IBZM was prepared [M.-P. Kung and H. F. Kung, *J. Labelled Comps. Radiopharm.* **27**, 691 (1989)] and administered intravenously (mean dose: 5.27 ± 0.77 mCi). Multiple SPECT scans [CERASPECT; Digital Scintigraphics, Waltham, MA; full width at half-maximum (FWHM), 11.5 mm; 64 1.67-mm slices; 15 min per scan] began 15 min after injection and continued over 4 hours. Individual scans were coregistered in three orthogonal planes to templates created on the 4-hour time-averaged image. To define anatomical ROIs, we also coregistered volume magnetic resonance imaging (MRI) scans (GE 1.5T Signa; spoiled gradient recalled acquisition in steady state; repetition time, 24 ms; time to echo, 5 ms) with the time-averaged image. ROIs sampling the head of the caudate nucleus, the body of putamen, and the cerebellum were drawn on five contiguous transverse MRI slices and transferred to corresponding SPECT images. Activity concentration (cpm/ml) was measured for the volume encompassed by each set of ROIs, corrected for decay, and normalized to injected dose.
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13. Individual caudate nucleus binding values by twin pair (more severe, less severe) were as follows: 1.51, 1.50; 1.67, 1.31; 1.12, 0.93; 1.19, 1.15; and 1.97, 1.34. Putamen values were as follows: 2.11, 2.77; 2.12, 2.32; 2.03, 1.60; 1.60, 1.43; and 2.27, 2.14.
14. Participants underwent a single 30-min SPECT scan (7.5 mm FWHM) beginning 15 min after intravenous administration of [^{99m}Tc]HMPAO (mean dose: 14.9 ± 0.2 mCi). Attenuation-corrected scans were coregistered and analyzed as described (*11*) except that these data were normalized to the whole slice. Striatal blood flow measurements revealed no significant differences between more and less affected twins for either caudate nucleus (mean ± SEM: 90 ± 3 compared with 88 ± 4) or putamen (127 ± 2 compared with 128 ± 2) (Wilcoxon test, $P = 0.69$ for both).
15. The time of peak specific [^{123}I]IBZM binding was estimated from the midpoint of the linear portion in the integral method (*12*) and ranged from 75 to 125 min after injection. Within-pair concordance on this measure was high [unbiased intraclass correlation coefficient, ICC(U), = 0.94 for caudate nucleus, $P < 0.0005$; ICC(U) = 0.82 for putamen, $P < 0.005$] [J. J. Bartko and W. T. Carpenter Jr., *J. Nerv. Ment. Dis.* **163**, 307 (1976)].
16. A statistical power analysis revealed that, given our means and standard deviations for caudate nucleus IBZM binding data, a sample size of 33 persons in each group would be required for 90% power to observe a significant difference in a nontwin design. A similar analysis for the putamen data revealed that more than 9000 persons in each group would be required for this region.
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KUZ, a Conserved Metalloprotease-Disintegrin Protein with Two Roles in *Drosophila* Neurogenesis

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During neurogenesis in *Drosophila* both neurons and nonneuronal cells are produced from a population of initially equivalent cells. The *kuzbanian* (*kuz*) gene described here is essential for the partitioning of neural and nonneuronal cells during development of both the central and peripheral nervous systems in *Drosophila*. Mosaic analyses indicated that *kuz* is required for cells to receive signals inhibiting the neural fate. These analyses further revealed that the development of a neuron requires a *kuz*-mediated positive signal from neighboring cells. The *kuz* gene encodes a metalloprotease-disintegrin protein with a highly conserved bovine homolog, raising the possibility that *kuz* homologs may act in similar processes during mammalian neurogenesis.

Neurogenesis in the fruit fly *Drosophila melanogaster* requires that cells from initially equivalent populations be selected to adopt different fates (*1*). In both the central and peripheral nervous systems, the selection of neural cells occurs in a stepwise process controlled by two groups of genes (*2*). First, genes of the proneural class confer equivalent neural potential on groups of cells. Subsequently, members of the neurogenic gene class ensure that only a single cell in each group is allowed to achieve its neural potential, whereas the others become epi-

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dermal. The emerging neural cell inhibits the neural potential of its neighboring cells in a process termed lateral inhibition (3). Loss-of-function mutations in proneural genes result in too few cells adopting a neural fate, whereas mutations in genes of the neurogenic class disrupt the mechanism of lateral inhibition and result in hyperplasia of the nervous system (2). We have identified and characterized a gene whose mutant phenotype indicates that it plays an essential role in the lateral inhibition process. We found that its product, a conserved member of the metalloprotease-disintegrin family, is required at different points in neurogenesis to both promote and inhibit the neural cell fate.

Because many genes important in neurogenesis would be lethal in a homozygous mutant state, we screened for genes involved in lateral inhibition with the use of FLP/FRT chromosomes to produce mutant clones in mosaic animals (4) and isolated several alleles of a gene we call *kuzbanian* (*kuz*) (5). Animals with *kuz* mutant clones exhibited clusters of sensory bristles (Fig. 1A) at positions where single sensory bristles would normally be observed. Separate sockets were often seen with individual bristles, and stimulation of mutant bristles in a reflex test elicited a leg cleaning response (6), indicating that mutant clusters contained multiple sensory bristles and not just multiple shafts. This multiple-bristle phenotype has been observed in clones mutant for several neurogenic genes such as *Notch* (*N*) and *shaggy* (*sgg*, also known as *zeste-white 3*), and it is indicative of a failure of lateral inhibition during the development of the peripheral nervous system (2, 7). Unlike the *N* phenotype, *kuz* clones did not produce ectopic bristles, indicating that *kuz* is not required for correct spacing between proneural

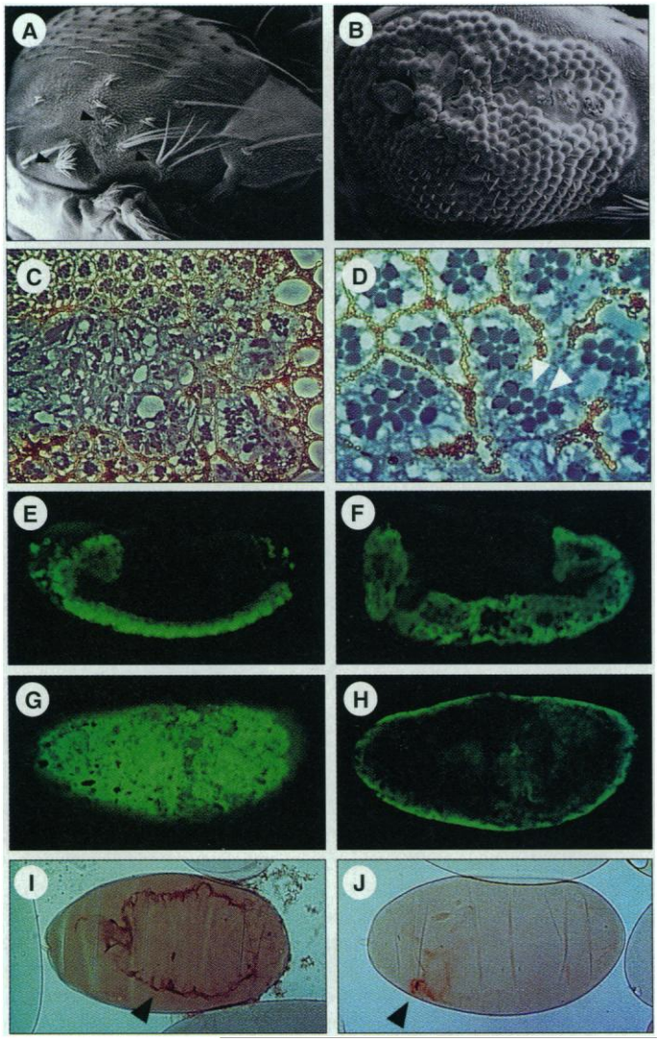

Fig. 1. The *kuzbanian* phenotype in adult clones and embryos (21). (**A**) Scanning electron micrograph (SEM) showing the multiple-bristle phenotype in an adult mosaic fly with homozygous *kuz*$^-$ clones. Several macro- and microchaete positions have supernumerary bristles (arrows), whereas others are missing in the same area. (**B**) SEM showing *kuz*$^-$ clones in the eye. The regular array of ommatidia is severely disrupted. (**C** and **D**) Sections through a mosaic eye. The *kuz*$^-$ clone is marked with *w*$^-$. Toward the center of the clone the density of photoreceptors is abnormally low, and none are successfully organized into ommatidia. The higher magnification view in (D) shows chimeric ommatidia at the clone border that contain a mixture of pigmented wild-type photoreceptor cells and mutant, unpigmented photoreceptors (arrowheads in one ommatidium). Confocal images in (E) to (H) of embryos stained with neuronal-specific anti-Elav demonstrate a requirement for maternal and zygotic *kuz* products. (**E**) Wild-type embryo, about stage 13, showing condensed neurons in the brain lobes and ventral nerve chord. Anterior is left and dorsal is up. (**F**) A *kuz* maternal null embryo with one zygotic copy of *kuz*. A greater proportion of the embryo has developed as neural tissue than in wild type. (**G**) The surface view of a *kuz* null embryo with no maternal or zygotic *kuz* product in which most cells have adopted a neural fate. In (**H**), a lower focal plane of this same embryo shows that all cells around the periphery of the embryo are neural cells. (**I**) Cuticular preparation of a *kuz* maternal null embryo with one zygotic copy of *kuz*. A small patch of cuticle develops (arrow) on the dorsal side of the embryo; presumably the remaining cells that failed to produce cuticle adopted a neural fate, consistent with the phenotype in (F). (**J**) Cuticular preparation of a *kuz* null embryo. Only a tiny dot of cuticle (arrow) has developed. Most of these embryos show no cuticle at all. A *kuz* null allele, *kuz*$^{e29\text{-}4}$, was used in all figures except (A) and (B), which were produced by *kuz*^{1} (5). Maternal null embryos were generated as in (22). Magnifications: (A) ×59; (B) ×118; (C) ×244; (D) ×783; and all the embryos in (E) through (J) are at approximately ×69.

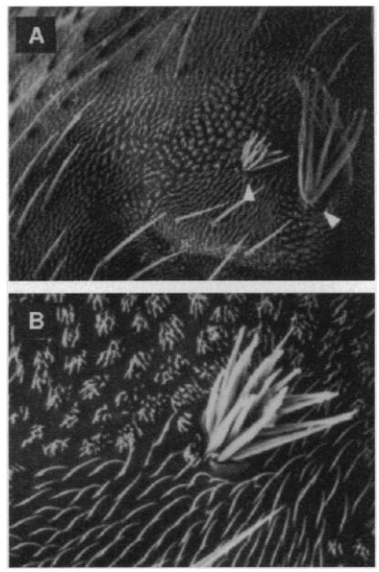

Fig. 2. Marked *kuz*$^-$ clones in the adult fly. (**A**) SEM of a *kuz*$^-$ clone. Each *kuz*$^-$ cell is also *ck*$^-$ and *y*$^-$. The *ck* mutation results in extra trichomes in the epidermal cell and in blunted shafts of sensory bristles; these morphological changes allow the border between mutant and wild-type cells to be precisely determined. There is a marked absence of all micro- and macrochaetes in the interior of the clone in (A), as no shafts, sockets, or neurons (naked cells) are seen. *kuz* mutant cells at normal bristle positions do form bristles at clone borders (arrowheads) where they are in contact with wild-type cells. (**B**) A high-magnification view of one of the multiple macrochaete clusters at a clone border. Every bristle in this and other clusters is always *ck*$^-$ and *y*$^-$ (the *y* marker cannot be scored in SEM), demonstrating that all bristles in a cluster are *kuz*$^-$. No wild-type bristles are observed in multiple-bristle clusters. Marked *kuz*$^-$ clones were generated in *y w hsFLP1; kuz*$^{e29\text{-}4}$ *ck P[FRT]40A/P[y*$^+$*] P[w*$^+$*] P[FRT]40A* first-instar larvae following protocols described in (4). Magnifications: (A) ×133; (B) ×405.

clusters. Mutant clones in the adult eye severely disrupted the regular array of ommatidia (Fig. 1B). Thin sections through such a mosaic eye revealed that mutant photoreceptors were not organized correctly into ommatidia (Fig. 1, C and D).

To determine whether *kuz* is required for the development of the central nervous system (CNS), we produced embryos lacking any maternally derived *kuz* product and containing one or no zygotic copies of *kuz*. We examined the embryos by staining with neuronal-specific antibodies to the Elav protein (anti-Elav) (*8*). Maternal null embryos with one copy of the zygotic *kuz* gene showed hyperplasia and disorganization of the CNS on the ventral side of the embryos (Fig. 1F), which is a phenotype similar to the neurogenic phenotype of *N* mutant embryos (*9*). However, embryos lacking all maternal and zygotic *kuz* product had a much more severe neurogenic phenotype. Hypertrophy of the nervous system was not restricted to the ventral region, but the embryos stained throughout with anti-Elav, demonstrating that many more cells in the embryo had developed as neural cells (Fig. 1, G and H). Such a severe neuralizing phenotype is similar to that of *sgg* null embryos (*10*). Cuticular preparations of embryos correlated well with the antibody results: Maternal-null embryos with one copy of *kuz* produced a small patch of cuticle on the dorsal side (Fig. 1I), consistent with the observation that many of the ventral cells had adopted a neural fate at the expense of epidermis. Embryos with no *kuz* product produced little or no cuticle (Fig. 1J), as would be expected if most cells had become neural, leaving few epidermal cells to secrete cuticle.

The observed phenotypes reveal that *kuz*

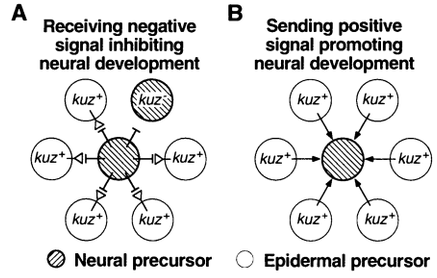

Fig. 3. The dual functions of *kuz* in bristle development. Mosaic analysis for *kuz*$^-$ clones in the adult cuticle indicates two distinct functions for *kuz*: (**A**) The failure of lateral inhibition, evidenced by the formation of extra bristles, only occurs in *kuz* mutant cells. This cell-autonomous mutant phenotype suggests that during normal development, the KUZ protein is required in cells to receive an inhibitory signal. (**B**) *kuz*$^-$ cells at normal bristle-forming positions become bristles only when they are in contact with wild-type cells, suggesting that in wild-type animals, KUZ may act as a positive signal or is involved in sending a positive signal for the development of the bristle. The predicted *kuz* gene product is a member of the metalloprotease-disintegrin family whose members undergo processing events, resulting in multiple functional peptides. KUZ may also undergo processing, and its proteolytic products may carry out different functions.

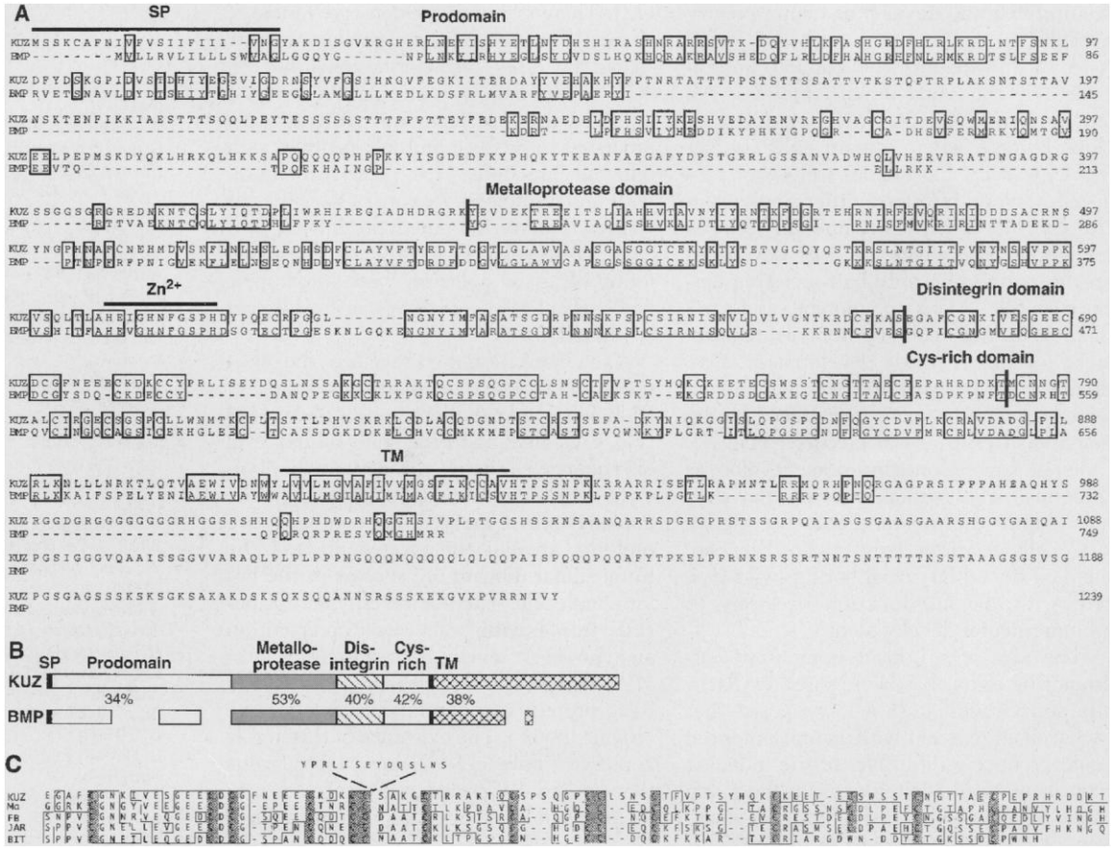

Fig. 4. Sequence and primary structure of the *kuzbanian* product. (**A**) Comparison of the predicted KUZ amino acid sequence with that of its closest homolog, a bovine metalloprotease (BMP) (*19*). Amino acid sequence of the *kuz* product was deduced from the nucleotide sequence of the 5.6-kb *kuz* NB1 cDNA. Identical amino acids are boxed. Functional domains as predicted by sequence and structural homology are labeled, including the zinc-binding site (Zn^{2+}) in the metalloprotease domain, the signal peptide (SP), and transmembrane domain (TM). (**B**) Similarity between KUZ and BMP. Percent amino acid identity between each domain is noted. Labeled domains correspond to sequences specified in (A). (**C**) Comparison of disintegrin domain of KUZ with those of meltrin-a (Ma), fertilin-β (Fβ), jararhagin (JAR), and bitistatin (BIT) (*22*). Consensus amino acids are boxed, and cysteine residues are shaded. At 110 amino acids, the KUZ disintegrin domain is the longest yet found. Its increased length is largely due to residues inserted between the sixth and seventh conserved cysteines. By comparison with the crystal structure of adamalysin (*23*), this region is predicted to form an unstructured loop maintained by disulfide bridges, and its expansion is not expected to disrupt the characteristic disintegrin secondary structure. As in many nonvenom disintegrin domains, the RGD residues are absent and an additional cysteine-containing sequence (ETEC) is observed. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

is involved in multiple developmental processes. We focused our subsequent analyses on the development of adult sensory bristles to determine a specific role for *kuz* in lateral inhibition. The *yellow* (y^-) and *crinkle* (ck^-) marker mutations were used to mark kuz^- clones in the adult cuticle. This allowed us to determine the genotype of individual cells and thus to examine the autonomy of the *kuz* mutant phenotype. Such analysis can distinguish between sending and receiving roles for a gene involved in the lateral inhibition process (*7*).

A role for *kuz* in lateral inhibition was suggested by the observation that all sensory bristles in a mutant cluster were kuz^-; no wild-type bristles were ever present in a cluster (Fig. 2B) (*11*). Thus, there is a cell-autonomous requirement for *kuz* in order for cells to be inhibited from adopting a neural precursor fate. A consistent explanation is that *kuz* is required in cells to receive an inhibitory signal from the emerging neural cell (Fig. 3). Cells in the proneural cluster with wild-type *kuz* function receive the inhibitory signal and are forced to become epidermal, whereas kuz^- cells cannot be inhibited and develop as neural precursor cells.

A second distinct role for *kuz* was revealed by the same mosaic analyses. All mutant bristle clusters were produced at clone borders, where mutant cells contact wild-type cells. No bristles were ever produced in clone interiors, either singly or in clusters (Fig. 2A) (*11*). Large kuz^- clones therefore caused bare patches devoid of bristles containing only hair-secreting epidermal cells. This phenotype indicates there is a non–cell-autonomous requirement for *kuz* in bristle development. One simple explanation is that a *kuz*-mediated neural-promoting signal is produced by the neighbors of the neural precursor (Fig. 3). Cells in kuz^- clone interiors develop as epidermal cells, perhaps because they cannot obtain the neural-promoting signal. kuz^- cells on clone borders, however, are supplied by their neighboring wild-type cells with the *kuz* function necessary to promote neural development.

The idea of a nonautonomous neural-promoting signal has been raised by Richelle and Ghysen (*12*) who proposed that each cell in a group with neural potential could produce a diffusible, bristle-inducing substance; a cell at the center of the group would be exposed to the highest concentration of the substance and would be most likely to go on to develop as a bristle. Stüttem and Campos-Ortega (*13*) have also presented evidence for a signal promoting neural development in transplantation studies in embryos. Their results showed that neuralizing signals from neighboring cells seem to be necessary for cells of the ectoderm to adopt a neural fate. *kuz* might be a component of this proposed neural-promoting signaling pathway. *kuz* apparently participates in both neural-promoting and -inhibiting processes during formation of the adult epidermis.

To reveal the molecular basis of the *kuz* functions, we cloned the *kuz* gene (*5*) and recovered a full-length cDNA (*14*) with an open reading frame that encodes a putative 1239–amino acid membrane-spanning protein of the metalloprotease-disintegrin family known as the ADAM family [members of the ADAM family contain a disintegrin and metalloprotease domain (*15*)] (Fig. 4, A and B). Although *kuz* is the only gene in this family whose loss-of-function phenotype has been characterized, properties of individual domains of ADAM proteins have previously been elucidated. Some members of the ADAM family are zinc-binding enzymes, and the KUZ metalloprotease domain also contains a conserved zinc-binding site (Fig. 4A) (*16*). Disintegrins have a characteristic spacing of cysteine residues that is required for their direct binding to integrin receptors (*17*). These cysteines are conserved in KUZ along with many additional residues that are shared by other disintegrin domains (Fig. 4C). In this family, many proteins with a multidomain structure are proteolytically processed to produce multiple peptides with different functions (*18*). The metalloprotease and disintegrin domains of KUZ may be cleaved from the full-length precursor to produce both soluble and membrane-bound forms of these domains. Such proteolytic products of KUZ could carry out the different *kuz* functions.

Data bank searches revealed the existence of a close mammalian homolog of KUZ, a bovine metalloprotease (BMP) (*19*) (Fig. 4A). KUZ and BMP share a high level of sequence similarity throughout the molecule (43% amino acid identity). All the domains are conserved between the two proteins, though the prodomain and the intracellular domain are shorter in the bovine molecule. The bovine enzyme was purified from bovine brain myelin preparation and can digest myelin basic protein in vitro (*19*), though it is not clear whether myelin basic protein is a natural substrate for the enzyme in vivo. The existence of this highly conserved bovine KUZ homolog suggests that similar KUZ-mediated processes may be involved in mammalian neurogenesis.

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FRT40A/ovo^{D} *FRT40A* females with germ-line clones to *y, w hsFLP; kuz*$^{e29\text{-}4}$ *ck FRT40A/CyO* males. About half of the embryos from these crosses exhibited the severe neurogenic phenotype described (Fig. 1G). The other half of the embryos exhibited an intermediate neurogenic phenotype (Fig. 1F), the same phenotype observed in all embryos produced by mating with wild-type males. The extent of the neurogenic phenotype caused by the hypomorphic *kuz* alleles varies between the wild type and the *kuz* null, but the correlation between the neural hyperplasia and the epidermal hypotrophy was always observed.

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Modulation of Virulence Factor Expression by Pathogen Target Cell Contact

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Upon contact with the eukaryotic cell, *Yersinia pseudotuberculosis* increased the rate of transcription of virulence genes (*yop*), as determined by in situ monitoring of light emission from individual bacteria expressing luciferase under the control of the *yopE* promoter. The microbe-host interaction triggered export of LcrQ, a negative regulator of Yop expression, via the Yop–type III secretion system. The intracellular concentration of LcrQ was thereby lowered, resulting in increased expression of Yops. These results suggest a key role for the type III secretion system of pathogenic bacteria to coordinate secretion with expression of virulence factors after physical contact with the target cell.

Human pathogenic *Yersinia* harbor a common 70-kb virulence plasmid encoding a set of highly regulated secreted proteins, Yops, that are key factors in the virulence process (*1–3*). When the bacteria are incubated under high Ca^{2+} conditions (≤1 mM) at 37°C, Yops are expressed at a low repressed level and no Yops are secreted to the culture medium. However, if Ca^{2+} is omitted, transcription is derepressed and high amounts of Yops are secreted to the culture medium (*1, 2, 4*). Thus, at the temperature of infection, Yop expression and secretion are regulated by external stimuli, which in vitro is constituted by the concentration of Ca^{2+}.

After infection of HeLa cells with *Yersinia pseudotuberculosis* (in vivo), the Yop effectors are found in the cytosol of the eukaryotic cell (*5–9*). This translocation process is polarized, and after intimate contact between the target cell and the pathogen has been established, the Yop effectors are secreted and translocated at the zone of contact between the bacterium and the target cell (*5–9*). Thus, the bacterium senses the contact with the eukaryotic cell surface and transmits a signal, focusing the secretion of Yop effectors to the zone of contact. How this cross talk between the eukaryotic cell and the bacterium is mediated is not known.

Yops are secreted by means of a type III secretion system (Ysc) (*10–12*), which has a high level of homology with the corresponding systems of *Salmonella* and *Shigella*. These systems are functionally conserved, allowing both the secretion and translocation of heterologous effector proteins across the target cell membrane (*12, 13*). Moreover, *Shigella* shows a target cell–induced response, manifested by the rapid release of the Ipa proteins to the culture medium (*14, 15*), and *Salmonella* rapidly induces the formation of new surface structures upon cell contact (*16*). Thus, these three human pathogens have a high degree of similarity with respect to the delivery of effector proteins attacking the target cells. Increased expression of effector proteins after microbe-host interaction has been suggested but has not yet been shown. Here, we provide evidence that physical contact between the pathogen and its target cell induces increased gene expression of virulence factors.

To determine whether Yop expression was elevated after cell contact, we measured the amount of YopE after infection of cultured HeLa cells with the wild-type strain YPIII(pIB102) of *Y. pseudotuberculosis* (Fig. 1). The total amount of YopE increased 16-fold when the bacteria made contact with the HeLa cells as compared with bacteria grown in cell culture medium alone (Fig. 1C). This increase was blocked by the addition of chloramphenicol (Fig. 1A). Similarly, the expression of the other Yop

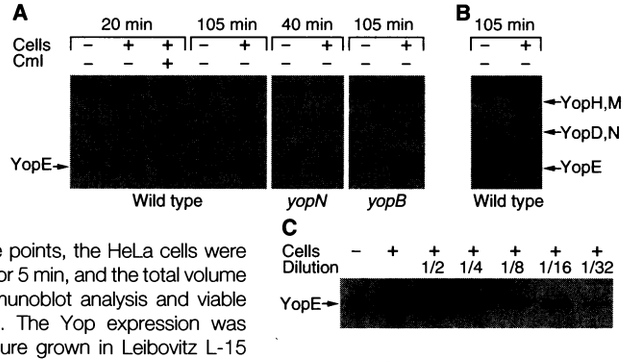

Fig. 1. HeLa cell contact stimulates increased expression of Yop proteins by *Y. pseudotuberculosis*. Confluent layers of HeLa cells in Leibovitz L-15 medium (cells +) (*24*) were infected with the wild type (YPIII/pIB102), a *yopN* mutant (YPIII/pIB82), or a *yopB* mutant (YPIII/pIB604). At the indicated time points, the HeLa cells were lysed with 0.1% Triton X-100 for 5 min, and the total volume was collected for protein immunoblot analysis and viable bacteria count determination. The Yop expression was compared with a control culture grown in Leibovitz L-15 medium alone (cells −). Protein samples corresponding to equal numbers of bacteria were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (12% gel) and transferred to nitrocellulose membranes (*25*). The blots were then probed with (**A**) a polyclonal antiserum to YopE or with (**B**) a polyclonal antiserum recognizing all Yops. (**C**) The concentrations of YopE 20 min after infection were quantified by analyzing serial dilutions of the samples (cells +). The blots were developed with an Amersham ECL-protein immunoblot kit according to instructions from the manufacturer. The amount of protein used for the blot in (C) was 10 times that used in (A). Cml = chloramphenicol.

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