failure to perform meiosis, accompanied by limited differentiation of tetraploid spermatids, has also been observed for mutations in two other *Drosophila* loci, namely *pelota* and *twine*[17–20]. The biochemical function of *pelota* has not been defined, but *twine* is known to encode the meiosis-specific homologue of Cdc25 phosphatase.

The locus *twine* is an established regulator of M-phase progression. The similarity in phenotype between *boule* and *twine* suggests that *boule*, and by analogy *DAZ* in humans, controls meiotic cell divisions. As putative RNA-binding proteins, Boule and DAZ might be involved in the processing, localization or translation of mRNA[7,8]. Although several other proteins in the RNP family have been implicated in the regulation of spermatogenesis in *Drosophila* and vertebrates, they are generally required during postmeiotic differentiation, a stage in which there is substantial translation of stored mRNAs[21,22].

We have shown that *boule* resembles *AZF* in loss-of-function phenotype, and *DAZ* in both testis-specific pattern of expression and amino-acid sequence. These findings provide strong support for the hypothesis that *DAZ* is *AZF*. Comparative studies of fertile and infertile men indicate that mutations in *DAZ* are extremely frequent, occurring at a frequency of at least 1 in 8,000 men, and that *DAZ* deletions contribute to cases of oligospermia, which is reduced sperm count, as well as azoospermia[5,23]. Future investigations into the function of *DAZ* and *boule* might provide further insight into both the regulation of the meiotic cell cycle and the physiological basis of a significant determinant of human infertility. □

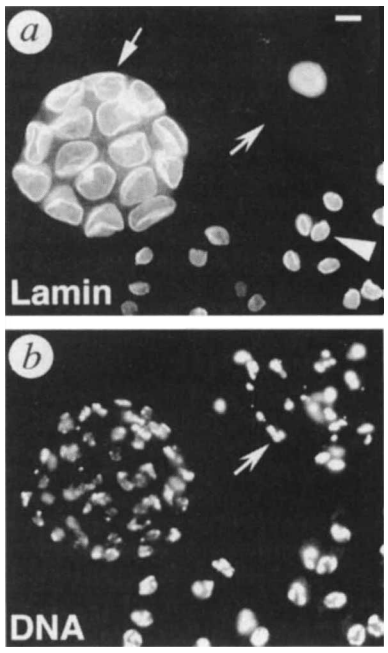

FIG. 4 Persistence of the nuclear lamina in *boule*. Fixed testes contents; spermatocytes and tetraploid spermatids from a *bol*^{1} homozygote. The images are paired, showing staining of the same region with either: *a*, antibodies to nuclear lamin; or *b*, the DNA-binding dye Hoechst. Scale bar, 10 μm. The nuclear lamina is clearly visible in mature *boule* spermatocytes (*a*, arrow). It is still intact in *boule* tetraploid spermatids (*a*, arrowhead) in which the mitochondria have begun to form the spermatid nebenkern. The nuclear lamina is eventually degraded, and is not present in older *boule* tetraploid spermatids (*a*, *b* notched arrows), although the lamina surrounding the large somatic cyst nucleus is still clearly visible (*a*, top right). METHODS. Sample preparation and analysis were as in Fig. 3. Monoclonal anti-lamin antibodies (from D. Glover) were used undiluted.

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CORRESPONDENCE and requests for materials should be addressed to S.A.W. (e-mail: stevenw@pooh.swmed.edu). The *boule* cDNA sequence has been deposited in Genbank under accession number U51858.

Functional receptor for GDNF encoded by the c-*ret* proto-oncogene

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GLIAL-CELL-LINE-DERIVED neurotrophic factor (GDNF) promotes the survival and phenotype of central dopaminergic[1,2], noradrenergic[3] and motor neurons[4–6], as well as various subpopulations of peripheral sensory and sympathetic neurons[7,8]. GDNF is structurally related to members of the transforming growth factor (TGF)-β superfamily[9], several members of which have well-characterized receptor systems[10,11]; however, GDNF receptors still remain undefined. Here we show that GDNF binds to, and induces tyrosine phosphorylation of, the product of the c-*ret* proto-oncogene, an orphan receptor tyrosine kinase, in a GDNF-responsive motor-neuron cell line. Ret protein could also bind GDNF and mediate survival and growth responses to GDNF upon transfection into naive fibroblasts. Moreover, high levels of c-*ret* mRNA expression were found in dopaminergic neurons of the adult substantia nigra, where exogenous GDNF protected Ret-positive neurons from 6-hydroxydopamine-induced cell death. Thus the product of the c-*ret* proto-oncogene encodes a functional receptor for GDNF that may mediate its neurotrophic effects on motor and dopaminergic neurons.

Because GDNF promotes the survival of primary motor neurons in culture and *in vivo*, we investigated the biological responses to GDNF in a motor-neuron hybrid cell line (hereafter called MN1) derived from embryonic mouse spinal motor neurons[12]. GDNF treatment of serum-deprived MN1 monolayers increased cell number in a dose-dependent manner (Fig. 1*a*). This biological response correlated with transient tyrosine phosphorylation of MAP kinases and elevated levels of c-*fos* mRNA (data not shown). The biological and biochemical responses to GDNF observed in MN1 cells indicated that these cells possess functional GDNF receptors. To identify GDNF binding components on MN1 cells, we chemically crosslinked ^{125}I-GDNF to MN1

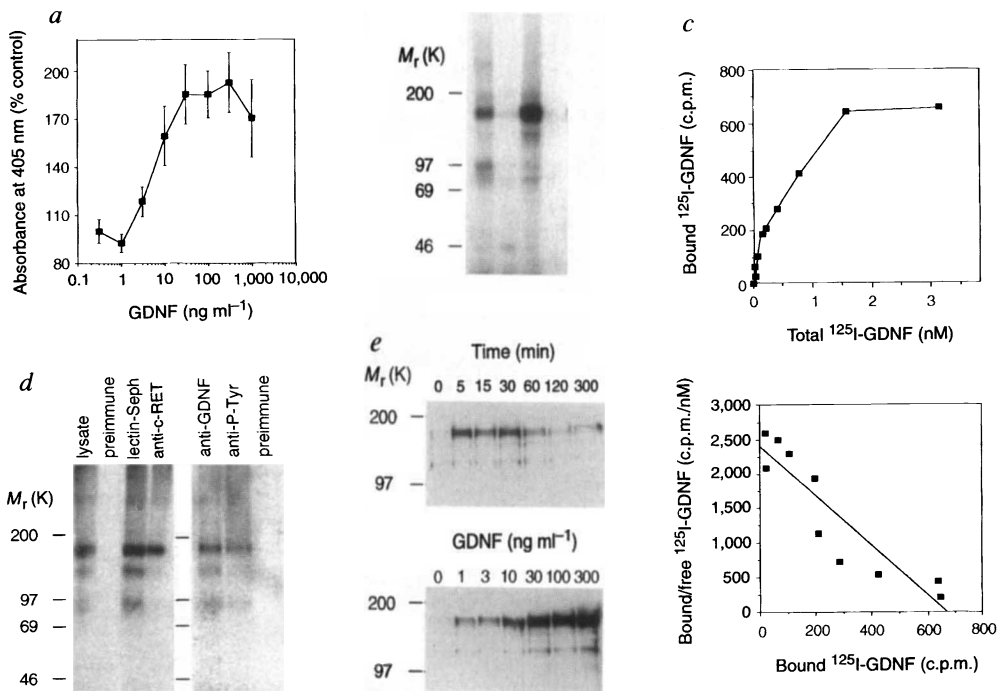

FIG. 1 Ret is a functional receptor for GDNF. *a*, GDNF stimulates survival of serum-deprived MN1 cells. *b*, Crosslinking of iodinated GDNF to receptors on MN1 cells. Note that the labelling could be displaced by excess cold GDNF. *c*, Equilibrium binding assay of GDNF to the 155K receptor on MN1 cells (top) and linear transformation of the data (bottom). A K_d of 2.18± 0.53 × 10^{-10} M was obtained in 4 independent experiments. The results of one representative experiment are shown. *d*, Immunoprecipitation analysis of GDNF–receptor complexes in MN1 cells. GDNF-labelled binding proteins could be precipitated with lectin–Sepharose beads, or antibodies against GDNF, phosphotyrosine (P-Tyr) and Ret. Control preimmune antibodies did not immunoprecipitate GDNF receptor complexes. *e*, GDNF induces tyrosine phosphorylation of Ret in MN1 cells. Ret tyrosine phosphorylation was detected 5 min after addition of GDNF (top). Saturation of Ret tyrosine phosphorylation was observed at 30 ng ml^{-1} GDNF (bottom).

METHODS. *a*, MN1 cell monolayers were exposed to increasing concentrations of GDNF in serum-free medium and assayed 3 days later for cell survival and growth by measurement of acid phosphatase activity (Clontech). GDNF was produced and purified from baculovirus-infected insect cells as previously described[7]. *b*, GDNF was iodinated as previously described[7] to a specific activity of 2 × 10^8 c.p.m. per μg. Iodinated GDNF at 10 ng ml^{-1} was allowed to bind to MN1 monolayers at 4 °C for 3 h, and was subsequently crosslinked using either DSS or EDAC as crosslinking agents. Unlabelled GDNF was used at 50× molar excess. Receptor complexes were fractionated by SDS–PAGE and visualized by autoradiography. *c*, Increasing concentrations of ^{125}I-GDNF were incubated with MN1 cell monolayers and, after 3 h incubation at 4 °C, receptor–ligand complexes were crosslinked with EDAC at 4 °C, fractionated by SDS–PAGE, and visualized by autoradiography. Gel bands corresponding to the major receptor complex of 180K were excised and quantified in a γ counter. Counts of GDNF bound to receptors were plotted as a function of the total amount of labelled GDNF added (top), and a linear transformation of these data was made in a Scatchard plot (bottom). A similar analysis was also done with the 95K receptor complex (not shown). *d*, ^{125}I-GDNF was crosslinked to MN1 cells using EDAC and receptor complexes were precipitated with antibodies against GDNF[7], lectin–Sepharose beads (Pharmacia), anti-phosphotyrosine antibodies (UBI), anti-Ret antibodies (Santa Cruz) or control antibodies from non-immune rabbits. *e*, MN1 cell monolayers were exposed to GDNF at different concentrations or for different periods of time and cell lysates were immunoprecipitated with anti-Ret antibodies and analysed by SDS–PAGE and western blotting with anti-phosphotyrosine antibodies.

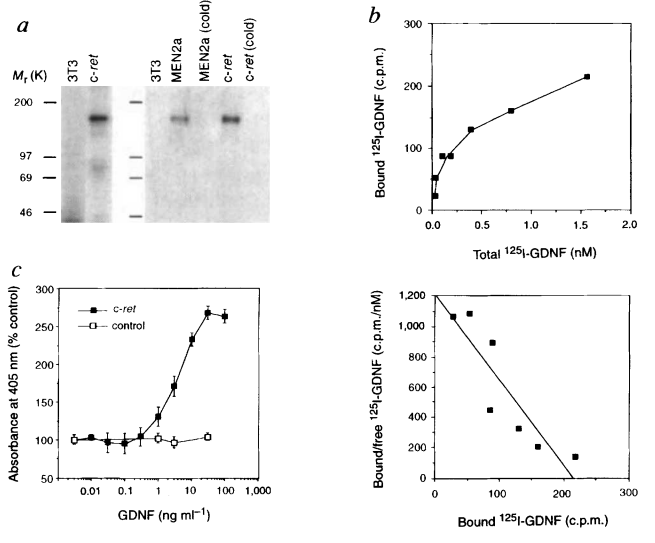

FIG. 2 Ret expression confers binding and biological responsiveness to GDNF in fibroblasts. *a*, Iodinated GDNF at 10 ng ml^{-1} could be crosslinked to 3T3 cells stably transfected with a wild-type c-*ret* expression plasmid (left). Untransfected 3T3 cells (3T3) did not bind GDNF. After immunoprecipitation with anti-Ret antibodies (right), GDNF–Ret complexes could be detected in 3T3 fibroblasts transfected with MEN2a-*ret* and wild-type c-*ret* expression plasmids, but not in untransfected cells. The specificity of the binding was demonstrated by displacement of the labelling with 50× excess unlabelled GDNF. *b*, Equilibrium binding assay of GDNF to Ret ectopically expressed on 3T3 fibroblasts (top) and Scatchard transformation of the data (bottom). A K_d of 2.75 ± 1.16 × 10^{-10} M was obtained in 3 independent experiments. The results of one representative experiment are shown. *c*, GDNF promotes survival and growth responses in 3T3 fibroblasts stably transfected with a c-*ret* expression plasmid. Untransfected cells did not respond to GDNF.

METHODS. For c-*ret* expression in transfected cells, human wild-type c-*ret* and MEN2a-*ret* cDNAs were subcloned in pcDNA3 (Invitrogen). The MEN2a-*ret* allele corresponded to the point mutation C634R. Cold GDNF was used at 50× molar excess. Saturation binding assays to Ret expressed on fibroblasts were performed as described in the Fig. 1 legend. For survival and growth assays, cells were cultured for 6 days in serum-free medium supplemented with the indicated concentrations of GDNF; medium and GDNF were replaced every 2 days. Cell survival and growth was quantified by measurement of acid phosphatase activity (Clontech).

monolayers and visualized the resulting complexes by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). A major complex of relative molecular mass 180,000 (M_r 180K) was crosslinked by ethyl-dimethylaminopropyl carbodiimide (EDAC) which approximately corresponds to a binding protein of 155K crosslinked to a glycosylated GDNF monomer (M_r ~25K) (Fig. 1*b*). An excess of cold ligand displaced ^{125}I-GDNF from this receptor complex, indicating that the labelling represented specific binding. The use of another crosslinker, disuccinimidyl suberate (DSS), also resulted in the labelling of this component, and of a minor component of 95K, corresponding to a binding protein of approximately 70K (Fig. 1*b*). The binding affinity of GDNF for its

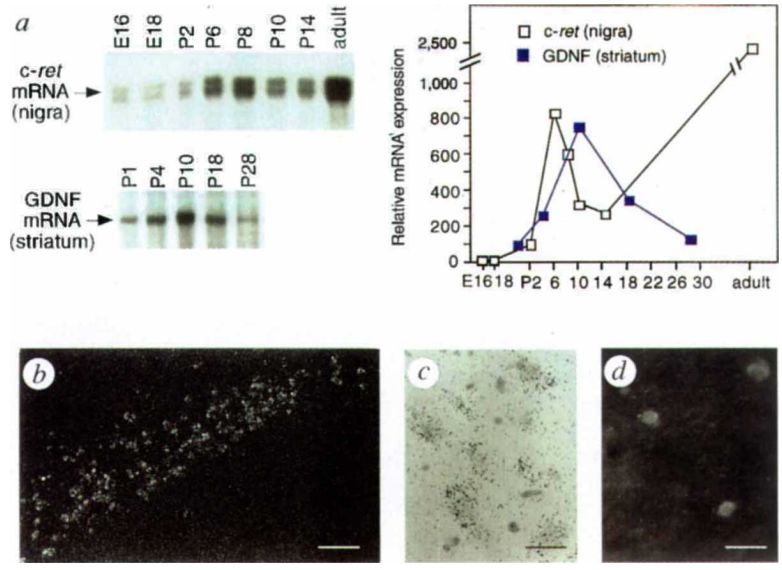

FIG. 3 Expression of c-*ret* mRNA in adult and developing substantia nigra. *a*, RNase protection assay (RPA) of c-*ret* mRNA expression during the development of the rat ventral mesencephalon (nigra), and of *GDNF* mRNA expression in the developing striatum. In the plot to the right, mRNA expression is indicated in arbitrary units where 100 corresponds to the level of expression in the respective regions in newborn animals. *b*, Dark-field autoradiogram of c-*ret* mRNA expression in the adult substantia nigra analysed by *in situ* hybridization. Scale bar, 40 μm. *c*, Bright-field autoradiogram showing substantia nigra neurons containing c-*ret* mRNA. Scale bar, 7.5 μm. *d*, Immunohistochemical analysis of Ret protein expression in the adult substantia nigra. Scale bar, 27 μm.

METHODS. A rat c-*ret* riboprobe was generated using as template a cDNA fragment obtained by PCR with primers based on sequences U22513 and U22514 (Genbank accession numbers), resulting in a 262-bp fragment from the extracellular domain of rat c-*ret*. For mRNA quantification, a glyceraldehyde-3-P dehydrogenase (GAPDH) riboprobe was included in the RPA, and values of relative mRNA expression, obtained after densitometric scanning of gel autoradiograms, were normalized using the GAPDH signal of each RNA sample. RPA for GDNF mRNA has been previously described[7]. *In situ* hybridization and immunohistochemistry were performed as previously described[23,24]. Ret protein was detected using a hamster monoclonal anti-mouse Ret antibody that also recognizes rat Ret[17], followed by fluorescein-conjugated rabbit anti-hamster secondary antibodies (Southern Biotechnologies).

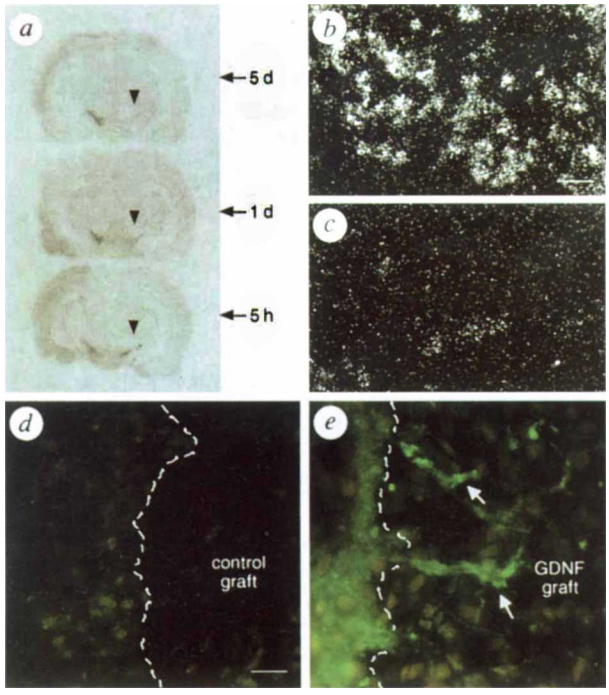

FIG. 4 Ret is expressed in GDNF-responsive substantia nigra dopaminergic neurons. *a*, Autoradiogram showing *in situ* hybridization for c-*ret* mRNA in the adult rat brain after a unilateral lesion with 6-OHDA. The injection of this toxic dopamine analogue in the medial forebrain bundle ensures that only cells which actively take up and retrogradely transport dopamine will be compromised. Note the reduction of the labelling for c-*ret* mRNA in the lesioned substantia nigra (arrowhead) 1 day and 5 days following the lesion. Dark-field autoradiograms (*b* and *c*) show c-*ret* mRNA expression at higher magnification in control (*b*) and lesioned (*c*) substantia nigra 5 days after unilateral injection of 6-OHDA. Scale bar, 25 μm. *d*, Immunohistochemical analysis of Ret protein expression in the adult substantia nigra after lesion with 6-OHDA and grafting of mock-transfected fibroblasts (control graft). The border of the transplant is outlined. Note the nearly complete absence of Ret-LI caused by the lesion. Scale bar, 20 μm. *e*, Grafting of GDNF-expressing fibroblasts rescues Ret-LI. Note Ret-positive fibres surrounding and entering the GDNF-producing graft (arrows). Magnification is as in *d*.

METHODS. Lesions of dopaminergic neurons of the substantia nigra were performed by stereotaxic injections of 8 μg 6-OHDA in the medial forebrain bundle at the following coordinates: 1.6 mm caudal to bregma; 1.3 mm lateral to midline; and 8.4 mm under the dural surface with the incisor bar 5 mm over the interaural line. Animals were pretreated with 25 mg per kg desipramine (intraperitoneal) 30 min before 6-OHDA injection. GDNF-expressing fibroblast cells (0.75×10^6) in 3 μl of medium were injected supranigrally at the following coordinates: 3.1 mm from interaural line; 2 mm lateral to midline; and 7 mm under the dural surface, with the incisor bar at −3.3 mm. The generation and characterization of GDNF-expressing fibroblasts have been described previously[3].

receptors in MN1 cells was assessed in a dose–response equilibrium binding assay (Fig. 1*c*). GDNF binding to either receptor component was saturable, and a linear transformation of these data indicated a dissociation constant. (K_d) of $2.18 \pm 0.53 \times 10^{-10}$ M (4 experiments) for the major 155 K receptor component (Fig. 1*c*), and of $6.67 \pm 3.01 \times 10^{-10}$ M (2 experiments) for the 70K component (data not shown). GDNF receptor complexes could be recovered by immunoprecipitation with anti-GDNF antibodies or by binding to lectin–Sepharose beads (Fig. 1*d*). Unexpectedly, the 180K receptor complex could also be recovered by immunoprecipitation with anti-phosphotyrosine antibodies (Fig. 1*d*), indicating that the GDNF binding protein in this complex could be a receptor tyrosine kinase.

The product of the c-*ret* proto-oncogene is a receptor tyrosine kinase that is highly expressed in primary motor neurons[13,14] and is of similar size to the major GDNF receptor component detected in MN1 cells[15]. Therefore, we tested whether the 180K band represented a Ret–GDNF crosslinked complex by immunoprecipitation with anti-Ret antibodies. An antipeptide Ret rabbit antiserum readily immunoprecipitated the major 180K ligand–receptor complex in MN1 cells (Fig. 1*d*), but several unrelated monoclonal and polyclonal antibodies used as controls failed to bring down this complex (Fig. 1*d*, and data not shown). We investigated whether GDNF could stimulate activation of the Ret receptor by examining tyrosine phosphorylation of the Ret protein in MN1 cells. GDNF treatment stimulated rapid Ret tyrosine phosphorylation in MN1 cells (Fig. 1*e*). Maximal phosphorylation was reached 5 min after GDNF treatment, and lasted for at least 60 min. A dose–response analysis of GDNF-induced Ret phosphorylation in MN1 cells showed maximal phosphorylation at 30 ng ml^{-1} GDNF (Fig. 1*e*), which is similar to the response of both serum-deprived MN1 cells (Fig. 1*a*) and embryonic sympathetic neurons[7] to GDNF.

We next examined whether expression of the c-*ret* gene product could allow binding of GDNF to cells unresponsive to the factor. To this end, GDNF binding and crosslinking experiments were performed with naive 3T3 fibroblasts, and 3T3 cells stably transfected with either a wild-type c-*ret* or an oncogenic form of this gene found in MEN2a patients[16]. Untransfected 3T3 fibroblasts did not bind GDNF at the concentrations tested, although, after transfection with a c-*ret* expression plasmid, iodinated GDNF strongly labelled a receptor component of 155K (Fig. 2*a*). After immunoprecipitation with Ret antibodies, GDNF–Ret complexes could be detected in both MEN2a-*ret* and c-*ret* transfected 3T3 fibroblasts, but not in untransfected cells (Fig. 2*a*). The labelling could be displaced by excess cold GDNF, indicating that it represented specific GDNF binding (Fig. 2*a*). The K_d of GDNF binding to Ret in transfected fibroblasts, as determined by equilibrium binding assay and Scatchard transformation, was $2.75 \pm 1.16 \times 10^{-10}$ M (3 experiments) (Fig. 2*b*), and was similar to that obtained in MN1 cells. In addition, very low levels of the 95K GDNF–receptor complex similar to that seen in MN1 cells could also be recovered. The fact that this component was affinity labelled by GDNF only after c-*ret* transfection suggests an interaction between these two receptor components. We also investigated whether c-*ret* could mediate a biological response to GDNF on transfection in non-responsive cells. Survival and growth responses to GDNF were investigated in untransfected and c-*ret* transfected 3T3 fibroblasts cultured in serum-free medium. GDNF elicited a dose-dependent increase in cell number in c-*ret* transfected, but not in untransfected, 3T3 cells (Fig. 2*c*), which was comparable to that previously observed in serum-deprived MN1 cells. Thus, although our data suggest the presence of additional GDNF-binding proteins, GDNF activity in 3T3 cells was seen only after c-*ret* transfection, indicating that c-*ret* expression imparts biological responsiveness to GDNF.

Having demonstrated that c-*ret* expression confers GDNF responsiveness, we investigated whether the c-*ret* product can mediate the neurotrophic effects of GDNF in the brain. We examined the expression of c-*ret* in different regions of the rat central nervous system. High c-*ret* mRNA expression was found in the adult rat spinal cord, pons, medulla, hypothalamus, thalamus and cerebellum (data not shown), but there were barely detectable levels in striatum, hippocampus and cerebral cortex (data not shown). In the ventral mesencephalon, which contains the cell bodies of GDNF-responsive dopaminergic neurons, c-*ret* mRNA levels increased progressively during postnatal development (Fig. 3*a*). A peak of expression was detected between postnatal day 6 (P6) and P8, at which time axons of dopaminergic neurons of the substantia nigra begin functional innervation of the striatum, and coincident with an increase in GDNF mRNA expression in this target region (Fig. 3*a*). *In situ* hybridization on sections through the adult substantia nigra revealed strong labelling over neurons throughout this structure (Fig. 3*b*, *c*). In addition, cells positive for Ret-like immunoreactivity (Ret-LI) were found throughout the adult substantia nigra, with strong labelling over cell bodies (Fig. 3*d*). To establish that c-*ret* expression in the adult substantia nigra was confined to dopaminergic neurons, we selectively lesioned these cells with a unilateral injection of 6-hydroxydopamine (6-OHDA), and analysed c-*ret* mRNA expression by *in situ* hybridization. No difference could be seen between ipsi- and contralateral sides in c-*ret* mRNA expression 5 h after the lesion (Fig. 4*a*). However, c-*ret* mRNA expression was markedly reduced in the lesioned substantia nigra one day after 6-OHDA treatment, and nearly absent five days after the lesion (Fig. 4*a*–*c*); c-*ret* mRNA expression in the side contralateral to the lesion was not affected. This indicated that, in the adult substantia nigra, c-*ret* mRNA expression was confined to dopaminergic neurons.

Finally, we investigated whether Ret-expressing neurons of the adult substantia nigra responded to GDNF. For this, we performed 6-OHDA lesions of nigral dopaminergic neurons, and examined whether grafts of GDNF-expressing fibroblasts induced responses on Ret immunoreactive cells. In lesioned rats that received a graft of control fibroblasts, no Ret-LI could be detected, indicating a depletion of Ret-expressing cells by selective lesion of dopaminergic neurons in the adult substantia nigra (Fig. 4*d*). However, Ret-LI could be rescued by the GDNF-expressing graft, where Ret immunopositive fibres could be seen surrounding and penetrating the transplant (Fig. 4*e*). The rescue of Ret-LI-positive cells and sprouting in the animals grafted with GDNF-expressing fibroblasts was similar to that of tyrosine hydroxylase immunoreactivity (data not shown), demonstrating that Ret-expressing adult dopaminergic neurons respond to GDNF.

Our results indicate that the Ret receptor tyrosine kinase is a signal-transducing receptor for GDNF. This finding is surprising, given that all receptors for members of the TGF-β superfamily characterized thus far are receptor serine-threonine kinases[10,11]. The ability of GDNF to interact with a receptor tyrosine kinase indicates a further functional divergence from other members of the TGF-β superfamily. Our results do not rule out the possibility that some of the activities of GDNF may be mediated by receptors with intrinsic serine-threonine kinase activity, however. Conversely, these findings could suggest that other TGF-β superfamily members may also use receptor tyrosine kinases. The expression of c-*ret* in cranial ganglia, autonomic and sensory neurons, and in several regions of the central nervous system, has led to the hypothesis that Ret is a receptor for a neurotrophic factor required for differentiation or survival of certain lineages of the mammalian nervous system[13]. Based on the evidence presented here, we propose that GDNF could be one such factor. Ret is one of the earliest markers expressed by postmigratory neural crest cells[17]. In line with this, several subpopulations of neurons derived from the neural crest have been found to respond to GDNF, including sympathetic neurons and a subpopulation of sensory neurons in the dorsal root ganglia[7,8]. Because Ret is highly expressed in the enteric nervous system[13], and c-*ret*$^{-/-}$ mice lack enteric neurons[18], our data predict that GDNF, which is highly expressed in the gastrointestinal tract[7,19], could also be a crucial

signal for the development of enteric neurons. Germline mutations of c-*ret* have been found to contribute to developmental abnormalities in a fraction of cases of Hirschsprung's disease, a congenital intestinal aganglionosis[20,21]. Because additional loci for Hirschsprung's disease other than c-*ret* have been proposed[22], our results prompt examination of the possibility that some of these loci might correspond to the *GDNF* gene. Finally, our findings indicate that Ret may mediate the neurotrophic effects of GDNF in the central nervous system. The expression of c-*ret* in motor and dopaminergic neurons is in accordance with the biological activities of GDNF on these cells. In particular, the patterns of mRNA expression in the developing brain presented here support the notion that interactions between GDNF and Ret play a trophic role in the innervation of striatum by dopaminergic substantia nigra neurons. Moreover, the ability of GDNF to protect Ret-positive neurons of the substantia nigra from 6-OHDA-induced cell death suggests that Ret mediates survival responses in these cells. Interestingly, however, the high levels of c-*ret* mRNA expression found in the adult substantia nigra, at a time when GDNF is minimally expressed in the brain[3], suggest that cognate ligands for Ret other than GDNF may be present in the adult brain. The neuroprotective activities of GDNF have stimulated great interest in this factor as a candidate therapeutic agent for the treatment of Parkinson's disease. New approaches for the treatment of this and other neurodegenerative diseases may be developed by targeting Ret or the signalling pathway that is initiated when this receptor is activated. □

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GDNF signalling through the Ret receptor tyrosine kinase

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Mutational analysis in humans and mice has demonstrated that Ret, the product of the c-*ret* proto-oncogene, a member of the receptor tyrosine kinase (RTK) superfamily[1], is essential for development of the enteric nervous system and kidney[2–6]. Despite the established role of Ret in mammalian embryogenesis, its cognate ligand(s) is currently unknown. Here we demonstrate, by using a *Xenopus* embryo bioassay, that glial-cell-line-derived neurotrophic factor (GDNF)[7], a distant member of the transforming growth factor(TGF)-β superfamily, signals through the Ret RTK. Furthermore, using explant cultures from wild-type and Ret-deficient mouse embryos[4], we show that normal c-*ret* function is necessary for GDNF signalling in the peripheral nervous system. Our data strongly suggest that Ret is a functional receptor for GDNF, and that GDNF, in addition to its potential role in the differentiation and survival of central nervous system neurons[8–12], has profound effects on kidney organogenesis and the development of the peripheral nervous system.

Activation of mitogen-activated protein kinase (MAPK) in animal cap cells isolated from blastula-stage *Xenopus* embryos, which normally give rise to epidermal tissue, is necessary and sufficient to induce mesoderm formation[13–15]. Because RTKs are potent activators of MAPK[16], we considered that signalling through the Ret RTK would also lead to mesoderm formation in animal caps. To test this, *in vitro* synthesized RNA that encodes a constitutively active form of the Ret receptor (Ret^{C634R}, in which a cysteine residue is replaced by an arginine at position 634 in the extracellular domain)[17,18] was injected into one-cell stage *Xenopus* embryos. Mesoderm formation was then assayed in animal caps dissected from blastula-stage embryos. Animal caps from control (uninjected) embryos, or embryos expressing wild-type Ret, differentiated into atypical epidermis as shown by their spherical shape, lack of mesodermal tissues, and failure to express Xbra (Fig. 1*a–d*, *g*). However, most (~82%) of the caps expressing Ret^{C634R} underwent elongation movements (Fig. 1*e*) similar to those caused by the addition of basic fibroblast growth factor, which is known to induce mesoderm formation. By the tadpole stage, histological analysis showed that these caps contained various types of mesodermal tissues, including mesenchyme, muscle and pronephros (Fig. 1*f*). The formation of mesoderm in caps expressing Ret^{C634R} was confirmed by the induction of Xbra RNA, a widespread mesodermal marker of early gastrula *Xenopus* embryos (Fig. 1*g*)[19]. These findings indicate that activation of the Ret signal transduction pathway is sufficient to induce formation of mesoderm in animal cap explants, and led us to postulate that coexpression of the wild-type Ret receptor and its putative ligand(s) would also lead to mesoderm formation.

GDNF is a distant member of the TGF-β superfamily, with potent neurotrophic effects on dopaminergic and motor neurons[7,10–12]. Although the *in vivo* function of GDNF is at present unclear, recent expression studies[20,21] have suggested a role in mammalian organogenesis, in particular the development of the kidney and gut, the two embryonic regions expressing the highest levels of GDNF mRNA. Because the *ret.k*$^-$ mutation in mice leads to characteristic defects in the development of the nervous system of the gut and kidney[4], we postulate that Ret might be a functional receptor for GDNF. To test this hypothesis, we assayed the competence of these molecules to induce mesoderm formation