cells is summed. In this model, all of the overlapping starburst cells would excite the ganglion cells; for geometric reasons, the summed input to the DS cell would be greatest when the stimulus lies directly over the dendritic field of the DS ganglion cell. Such a mechanism seems implausible: a broad annulus presented around the DS cell's dendritic field would stimulate the ganglion cell because of the summed outputs of the neighboring starburst cells. (iv) Inhibitory inputs [from other retinal neurons or from the starburst cells' own release of γ-aminobutyric acid (GABA) (*14*)] could somehow negate the laterally conducted effects of the overlapping starburst cells. (v) The starburst cells could play a role other than the transmission of the information about moving stimuli. Whether one of these or some other possibility is correct, the discussion must clearly be reopened. Our results, together with those of Bloomfield (*12*), challenge the validity of all current models of retinal directional selectivity (*15*).

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8. The methods used were those developed by Ames and colleagues [A. Ames and F. B. Nesbett, *J. Neurochem.* **37**, 867 (1980); (*5*)]. For injection of Fast blue, the rabbit was anesthetized with a combination of Nembutal and ether, the eye was protopsed, and 10 μl of 1% Fast blue was injected into the nerve through a slit in the conjunctiva. For isolation of the retina, the animal was again anesthetized, the eye removed, and the animal killed by an overdose of Nembutal without waking from anesthesia. The eye was everted under Ames medium, and the retina gently teased away from the pigment epithelium. Subsequent incubation and maintenance in vitro were as described (*5*).
9. This method combines the advantages of extracellular recording (intracellular recordings in rabbit retina are difficult and brief) with identification of the recorded cell, which usually requires intracellular recordings. It can also be used for the study of infrequently occurring types of cells in retina or in other neural tissues because it solves the problem of sampling bias that may occur with metal electrodes [W. R. Levick and L. N. Thibos, *Prog. Retinal Res.* **2**, 267 (1983)].
10. The boundaries estimated by flashing spots were nearly the same as those established with moving spots (in the preferred direction). The roll-off at the edges of a DS cell's receptive field is apparently sharper than that of the α and β cells [L. Peichl and H. Wassle, *J. Physiol.* **291**, 117 (1979)], making it relatively easy to determine the receptive field boundaries in DS cells. The boundary described here is for small spots flashed within the receptive field or moved across its edges. Under special conditions, an inhibitory region extending beyond the on-off zone of these cells has been demonstrated (*1*, *2*).
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15. A model by Borg-Graham [thesis, Massachusetts Institute of Technology (1991)] points out that many amacrine cells can have the asymmetry necessary for a DS input to the ganglion cells. To carry out this function, however, the postulated non-starburst amacrine must (i) have a density and dendritic spread similar to that of the starburst cell; (ii) lie on a direct excitatory pathway to the DS cell; (iii) generate no action potentials; and (iv) have a suitably short electrotonic length constant. No other known amacrine cell meets even the first two of these requirements.

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Cloning of a Delta Opioid Receptor by Functional Expression

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Opiate drugs have potent analgesic and addictive properties. These drugs interact with receptors that also mediate the response to endogenous opioid peptide ligands. However, the receptors for opioids have eluded definitive molecular characterization. By transient expression in COS cells and screening with an iodinated analog of the opioid peptide enkephalin, a complementary DNA clone encoding a functional δ opioid receptor has been identified. The sequence shows homology to G protein–coupled receptors, in particular the receptors for somatostatin, angiotensin, and interleukin-8.

Opiate drugs such as morphine affect the perception of pain, consciousness, motor control, and autonomic function by interacting with specific receptors expressed throughout the central and peripheral nervous systems (*1*). The endogenous ligands of these opiate receptors have been identified as a family of more than 20 opioid peptides that derive from the three precursor proteins proopiomelanocortin, proenkephalin, and prodynorphin (*2*). Although the opioid peptides belong to a class of molecules distinct from the opiate alkaloids, they share common structural features including a positive charge juxtaposed with an aromatic ring that is required for interaction with the receptor (*3*).

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Classical pharmacological studies have defined three classes of opioid receptors—δ, μ, and κ that differ in their affinity for various opioid ligands and in their distribution (*4*). The δ receptors bind with the greatest affinity to enkephalins and have a more discrete distribution in the brain than either μ or κ receptors, with high concentrations in the basal ganglia and limbic regions. Although morphine interacts principally with μ receptors, peripheral administration of this opiate induces release of enkephalins (*5*). Thus, enkephalins may mediate part of the physiological response to morphine, presumably by interacting with δ receptors. Despite pharmacological and physiological heterogeneity, the three types of opioid receptors inhibit adenylyl cyclase, increase K^+ conductance, and inactivate Ca^{2+} channels through a pertussis toxin–sensitive mechanism. These results and others suggest that opioid receptors belong to the large family of cell surface receptors that signal through G proteins (*6*).

Previous reports have described the isolation of two cDNAs that may function as opiate receptors. The sequence of a cDNA

encoding a protein that binds morphine shows homology to the phosphatidylinositol-linked members of the immunoglobulin superfamily but does not show the anticipated homology to G protein–coupled receptors (7). A putative κ receptor cDNA does show homology to G protein–coupled receptors but confers low-affinity binding to the alkaloid bremazocine and lacks the expected ligand selectivity (8).

To isolate a clone for the δ opioid receptor, we prepared a cDNA library from NG108-15 cells, which express approximately 300,000 δ receptors per cell (9), and transfected the library into COS cells. Screening with ^{125}I-labeled [D-Ala^2, D-Leu^5]-enkephalin (DADLE) identified one cluster of positive cells (10). After extraction of the DNA from these cells and two rounds of purification and retransfection, an individual cDNA (DOR-1) was isolated. Using an autoradiographic assay, we determined that the binding of ^{125}I-labeled

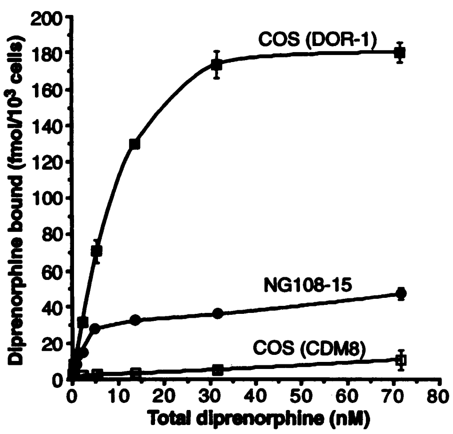

Fig. 1. Binding of [^{3}H]diprenorphine to intact NG108-15 cells, COS cells transfected with DOR-1 [COS (DOR-1)], and COS cells transfected with the CDM8 vector [COS (CDM8)] (18).

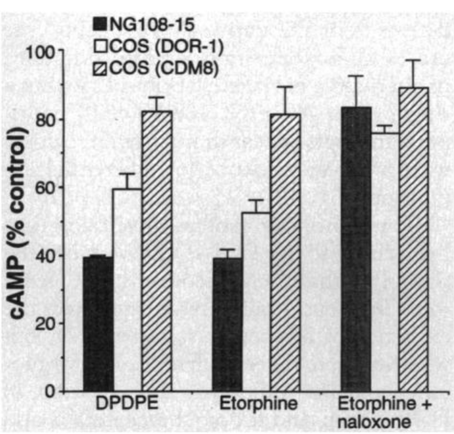

Fig. 2. Agonists for the δ opiate receptor reduce cAMP accumulation in cells transfected with DOR-1. NG108-15 cells and COS cells transfected with either DOR-1 [COS (DOR-1)] or the vector control [COS (CDM8)] were stimulated with 5 μM forskolin in the presence of 1 mM 3-isobutyl-1-methyl xanthine and 500 nM DPDPE or 100 nM etorphine. Cyclic AMP was assayed after 8 min at 37°C (Diagnostic Products, Los Angeles, California). Naloxone (100 μM) inhibited the decrease in cAMP stimulation observed in NG108-15 cells and COS (DOR-1) cells but not the relatively small reduction in cAMP levels seen in COS cells transfected with CDM8 vector alone.

A

```
                                                  * 20          *     40
DOR-1                        --MELVPSARAELQSSPLVNLSDAFPSAFPSAGANASGSPGARS------
SOMATOSTATIN RECEPTOR        MFPNGTASSPSSSPSPSPGSCGEGACSRGPGSGAADGMEEPGRN------
ANGIOTENSIN RECEPTOR         ---------------------------MALNSSAEDGIKRIQDDC------
IL8 RECEPTOR                 ------------------MSNITDPQMWDFDDLNFTGMPPADEDY------
FORMYL PEPTIDE RECEPTOR      ---------------------------------METNFSTPLNEY------
OPIOID BINDING RECEPTOR      ----MASPAGNLSAWPGWGWPPPAALRNLTSSPAPTASPSPAPSWTPSPR
                          60                   80                  100
DOR-1   AS-------SLALAIAITALYSAVCAVGLLGNVLVMFGIVRYTKLKTATNIYIFNLALADALATSTLPFQ
SOMAT   ASQNGTLSEGQGSAILISFIYSVVCLVGLCGNSMVIYVILRYAKMKTATNIYILNLAIADELLMLSVPFL
ANG     PKAGRHS--YIF--VMIPTLYSIIFVVGIFGNSLVVIVIYFYMKLKTVASVFLLNLALADLCFLLTCPLW
IL8     SPCMLET--ETLNKYVVIIAYALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLPIW
F-PEP   EEVSYESAGYTVLRILPLVVLGVTFVLGVLGNGLVIWVAGF-RMTRTVTTICYLNLALADFSFTATLPFL
OPB-R   PGPAHPFLQPPWAVALWSLAYGAVVAVAVLGNLVVIWIVLAHKRMRTVTNSFLVNLAFADAAMAALNALV
                  120                 140                 160
DOR-1   SAKYLMET-WPFGELLCKAVLSIDYYNMFTSIFTLTMMSVDRYIAVCHPVKALDFRTPAKAKLINICIWV
SOMAT   VTSTLLRH-WPFGALLCRLVLSVDAVNMFTSIYCLTVLSVDRYVAVVHPIKAARYRRPTVAKVVNLGVWV
ANG     AVYTAMEYRWPFGNHLCKIASASVTFNLYASVFLLTCLSIDRYLAIVHPMKSRLRRTMLVAKVTCIIIWL
IL8     A--ASKVNGWIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHLV-KFVCLGCWG
F-PEP   IVSMAMGEKWPFGWFLCKLIHIVVDINLFGSVFLIGFIALDRCICVLHPVWAQNHRTVSLAMKVIVGPWI
OPB-R   NFIYALHGEWYFGANYCRFQNFFPITAVFASIYSMTAIAVDRYMAIIDPLKPRL--SATATRIVIGSIWI
             180                 200                           220
DOR-1   LASGVGVPIMVMAVTQP-RDGAV-VCMLQFPS----------PSWYWDTVTKICVFLFAFVVPILIITVC
SOMAT   LSLLVILPIVVFSRTAANSDGTV-ACNMLMPE----------PAQRWLVGFVLYTFLMGFLLPVGAICLC
ANG     MAGLASLPAVIHRNVYFIENTNITVCAFHYESRNST----LPIGL------GLTKNILGFLFPFLIILTS
IL8     LSMNLSLPFFLFRQAYHPNNSS-PVCYEVLGNDTAK----WRMVL------RILPHTFGFIVPLFVMLFC
F-PEP   LALVLTLPVFLFLTTVTIPNGD-TYCTFNFASWGGTPEERLKVAITMLTARGIIRFVIGFSLPMSIVAIC
OPB-R   LAFLLAFPQCLYSKIKVMPGRTLCYVQWPEGSRQ-------------HFTYHMIVIVLVYCFPLLIMGIT
             240                 260                     280
DOR-1   YGLMLLRLRSVRLL----SGSKEKDRSLRRITRMVLVVVGAFVVCWAPIHIFVIVWTLV------DINRR
SOMAT   YVLIIAKMRMVALK----AGWQQRKRSERKITLMVMMVVMVFVICWMPFYVVQLVNVFA------E---Q
ANG     YTLIWKALKKAYEI----QKNKPRNDDIFRI---IMAIVLFFFFSWVPHQIFTFLDVLIQLGVIHD-CKI
IL8     YGFTLRTLFKAHMG----QKHRA-----MRV---IFAVVLIFLLCWLPYNLVLLADTLMRTQVIQETCER
F-PEP   YGLIAAKIHKKGMI----KSSRP-----LRV---LTAVVASFFICWFPFQLVALLGTVWLKEML--FYGK
OPB-R   YTIVGITLWGGEIPGDTCDKYQEQLKAKRKVVKMMIIVVVTFAICWLPYHIYFILTAIYQQL------NR
             300                320                   340
DOR-1   DPLVVAALHLCIALGYANSSLNPVLYAFLDENFKRCFRQ---LCR-TPCGRQEPGSLRRPRQATTRERVT
SOMAT   DDATVS--QLSVILGYANSCANPILYGFLSDNFKRSFQRILCLSW-MDNAAEEPVDYYATALKSRAYSVE
ANG     SDIVDTAMPITICIAYFNNCLNPLFYGFLGKKFKKYFLQLLKYIP-PKAKSHSSLSTKMSTLSYRPSDNM
IL8     RNNIGRALDATEILGFLHSCLNPIIYAFIGQNFRHGFLKILAMHG-LVSKEF--LARHRVTSYTSSSVNV
F-PEP   YKIIDILVNPTSSLAFFNSCLNPMLYVFVGQDFRERLIHSLPTSL-ERALSEDSAPTNDTAANCASPPAE
OPB-R   WKYIQQVYLASFWLAMSSTMYNPIIYCCLNKRFRAGFKRAFRWCPFIHVSSYDELELKATRLHPMRQSSL
         360         370
DOR-1   ACTPSDGPGGG--AAA-------------------------------------------------
SOMAT   DFQPENLESGGVFRNGTCASRISTL----------------------------------------
ANG     SSSAKKPASCFEVE---------------------------------------------------
IL8     SSNL-------------------------------------------------------------
F-PEP   TELQAM-----------------------------------------------------------
OPB-R   YTVTRMESMSVVFDSNDGDSARSSHQKRGTTRDVGSNVCSRRNSKSTSTTASFVSSSHMSVEEGS
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B

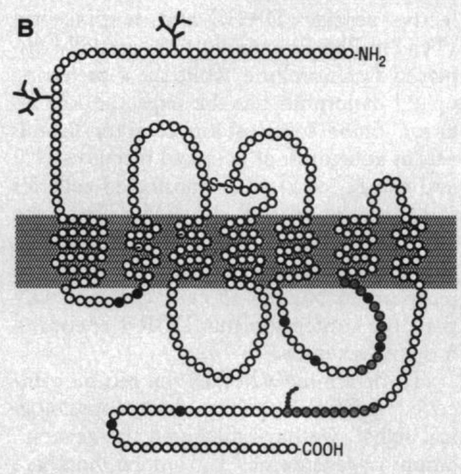

Fig. 3. (**A**) Comparison of DOR-1 with the mouse somatostatin receptor, rat angiotensin receptor, human interleukin-8 receptor, human *N*-formyl peptide receptor, and a putative κ opioid binding receptor (8). Identical amino acids are indicated in bold type. Seven predicted transmembrane domains are underlined. Consensus sequences for N-linked glycosylation in the predicted extracellular domains are shown with an asterisk. The nucleotide sequence for DOR-1 has been submitted to GenBank under accession number L07271. (**B**) Proposed structure of DOR-1. Potential sites for extracellular asparagine-linked glycosylation (branched structures) and cytoplasmic cysteine-linked palmitoylation (beaded structure) are shown at the NH$_2$-terminal and COOH-terminal domains, respectively. Solid circles indicate intracellular consensus sites for phosphorylation by cyclic nucleotide-dependent kinases and protein kinase C. Two predicted amphiphilic helical structures (shaded circles) occur in the third cytoplasmic loop and the COOH-terminus. A disulfide bridge may link the second and third extracellular domains and aspartate residues (−) occur in the second and third transmembrane domains.

Table 1. DOR-1 confers δ-selective high-affinity binding of [^{3}H]diprenorphine to transfected COS cell membranes (*19*).

Ligand	K_d (nM)
Opiate alkaloid ligands	
Diprenorphine	3.8
Etorphine	13.5
Levorphanol	107
Morphine	1,100
Dextrorphan	>50,000
Opiate peptide ligands	
DADLE	4.8
TIPP	5.4
DSLET	2.3
DPDPE	17.5
Dynorphin 1-17	239
DAGO	>50,000
Morphiceptin	>50,000
Somatostatin 14	>50,000
Des-Tyr-DADLE	>50,000

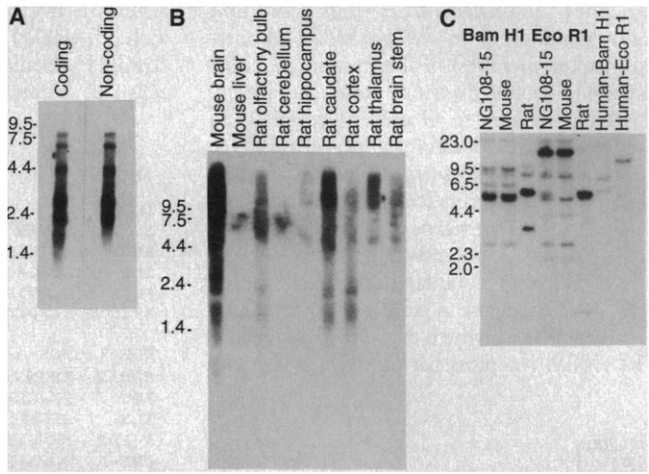

Fig. 4. Northern and Southern blot analysis of DOR-1. (**A**) A Northern blot of polyadenylated RNA (5 μg) from NG108-15 cells hybridized with either the complete protein-coding or the 3′ noncoding region of DOR-1 (*19*) shows multiple transcripts with both probes. (**B**) A Northern blot containing polyadenylated RNA (10 μg) from dissected mouse and rat tissues hybridized with the protein-coding region of DOR-1 shows multiple transcripts in different brain regions but no signal in the liver (*19*). (**C**) Southern blot analysis of DNA isolated from NG108-15 cells, mouse, rat, and human tissue with the entire DOR-1 cDNA as probe shows a single strongly hybridizing and multiple weakly hybridizing species from each source (*19*). NG108-15 cells contain a δ receptor gene from the mouse but not the rat parent used to produce this hybrid cell line (*9*).

DADLE to COS cells transfected with DOR-1 can be competed by nanomolar concentrations of the alkaloids diprenorphine, morphine, etorphine, and levorphanol and the peptides DADLE, [D-Ser^2]-Leu enkephalin-Thr (DSLET) and D-penicillamine^{2,5}-enkephalin (DPDPE). High concentrations (>5 μM) of dextrorphan (the inactive enantiomer of levorphanol) and the μ-selective peptide [D-Ala^2, *N*-methyl Phe^4, Gly^5-ol]-enkephalin (DAGO) do not compete with DADLE.

Intact COS cells transfected with DOR-1 also bound to the opiate antagonist [^{3}H]diprenorphine with high affinity (Fig. 1), and membranes from these cells showed considerable ligand specificity (Table 1). In addition to the δ-selective peptide agonists DSLET and DPDPE, the antagonist Tyr-tetrahydroisoquinoline-3-carboxylic acid-Phe-Phe (TIPP) competed for diprenorphin binding sites with high affinity [dissociation constants (K_d's) in the low nanomolar range]. In contrast, dextrorphan, the inactive peptide des-Tyr-DADLE, and the μ-selective peptides DAGO and morphiceptin (Tyr-Pro-Phe-Pro amide) only partially displaced diprenorphine, while the κ-preferring opioid dynorphin has the expected low affinity. Some somatostatin receptor ligands acts as antagonist at μ opioid receptors (*11*) and the K_d of DOR-1 transfected cells for somatostatin exceeds 10 μM. Thus, the selectivity of DOR-1 for different ligands agrees with values obtained for native δ receptors in NG108-15 cells (*12*) and supports the contention that DOR-1 encodes a δ opioid receptor.

To determine whether the receptor encoded by DOR-1 can transduce a physiological signal, we have measured the accumulation of adenosine 3′,5′-monophosphate (cAMP) in transfected cells (Fig. 2). Both the alkaloid etorphine and the peptide DPDPE reduced the forskolin-stimulated increase of cAMP by approximately 40%. The antagonist naloxone blocked this reduction. Thus, DOR-1 confers functional coupling of opioid ligands to the inhibition of adenyl cyclase with the appropriate pharmacology.

The nucleotide sequence of DOR-1 predicts a protein of 372 amino acids with considerable homology to G protein–coupled receptors (Fig. 3). As with many of these receptors, two consensus glycosylation sites occur in the NH_2-terminal domain of DOR-1 and a pair of cysteine residues proposed to form a disulfide bond occur in the first two extracellular loops (*13*). By similar analogy, the second and third transmembrane domains contain aspartate residues that may provide a counterion for the amino group of the ligand and confer the guanosine triphosphate (GTP) sensitivity of agonist binding (*13*). Further, the COOH-terminal domain of the protein contains a number of cysteine residues that may undergo palmitoylation (*13*). Multiple consensus sequences for phosphorylation appear on several cytoplasmic domains and may mediate the observed regulation of receptor function in response to continued opiate exposure (*14*).

The δ receptor shows somewhat greater homology to the receptors for somatostatin (37% identity), angiotensin (31% identity), and the chemotactic factors interleukin-8 (22% identity) and *N*-formyl peptide (21% identity) than to the putative κ receptor (8) (Fig. 3). All these receptors bind to peptide ligands and some of these, like opioids, elicit chemotaxis (*15*). The close homology of DOR-1 with the receptor for somatostatin has particular significance. Somatostatin and analogs of somatostatin bind to opioid receptors (*11*). Furthermore, opioid receptors and somatostatin receptors inhibit neurotransmitter release by reducing Ca^{2+} currents and increasing K^+ conductance, and both receptors couple to these ion channels through the same G_α subunit (*16*).

Northern blots of RNA from NG108-15 cells show multiple transcripts (Fig. 4A), raising the possibility that this probe hybridizes to the mRNA for related receptors. However, a fragment derived from the 3′-untranslated region of DOR-1 recognizes all but the shortest of these transcripts and hybridization of DOR-1 cDNA to genomic Southern blots reveals a single strongly hybridizing species (Fig. 4C). Thus, the heterogeneity of hybridizing species may not reflect multiple genes but presumably derives from alternative RNA splicing, the use of alternative transcription initiation, or alternative polyadenylation sites within a single gene. As in NG108-15 cells, multiple transcripts appear in most brain regions, with no clear evidence for differential expression (Fig. 4B).

The identification of multiple transcripts hybridizing to the DOR-1 cDNA raises the possibility that these encode distinct receptors. Previous studies have suggested the existence of δ receptor subtypes (*17*), but whether these reflect different receptor proteins remains unknown. The relation of DOR-1 to μ and κ opioid receptors is also of considerable interest. The presence of the multiple transcripts in the NG108-15 cells, which do not express μ or κ receptors, makes it unlikely that these transcripts account for the various opioid receptor classes. Thus, the other opioid receptors probably originate from distinct genes.

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10. A random primed, size-selected cDNA library from NG108-15 cells was prepared in the plasmid expression vector CDM8 [A. Aruffo and B. Seed, *ibid.* **84**, 8573 (1987)] and transfected into COS cells by electroporation. After 3 days, the cells were screened for opioid binding with 10 to 20 nM mono-^{125}I-labeled DADLE (at an approximate specific activity of 2000 Ci/mmol) that had been purified by high-performance liquid chromatography [R. J. Miller, K. J. Chang, J. Leighton, P. Cuatrecasas, *Life Sci.* **22**, 379 (1978); N. T. Maidment *et al.*, *Neuroscience* **33**, 549 (1989)]. After incubation for 1 hour in modified Krebs Ringer buffer (KRB) containing 1% albumin, the plates were washed rapidly with ice cold KRB and dried on ice with cold air. Plates were then exposed to Cronex film (Dupont), and, after alignment of the autoradiogram with the plates, the DNA was extracted from positive cells [B. Hirt, *J. Mol. Biol.* **26**, 365 (1967)] and electroporated into competent bacteria, and the DOR-1 plasmid isolated after two additional rounds of retransfection into COS cells.
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18. Binding to intact cells was carried out in modified KRB (*12*) for 20 min at room temperature and binding to membranes in 25 mM Hepes, 1% albumin, 5 mM $MgCl_2$, pH 7.7, for 1 hour at 4°C. Specific binding was defined by the displacement with diprenorphine.
19. RNA was isolated using the Fast Track mRNA Isolation Kit (Invitrogen), and Northern blot analysis performed at 65°C as described [T. G. Boulton *et al.*, *Cell* **65**, 663 (1991)], followed by autoradiography for 1 week at −70°C with an enhancing screen. Repeated studies consistently showed a wide range of transcripts, with no evidence for RNA degradation either by staining the gels with ethidium bromide or by rehybridization with a probe for β actin. Southern blots were washed in 0.2× standard sodium citrate, 0.1% SDS at 55°C.
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Prevention of Programmed Cell Death in *Caenorhabditis elegans* by Human *bcl-2*

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Programmed cell death is a physiological process that eliminates unwanted cells. The *bcl-2* gene regulates programmed cell death in mammalian cells, but the way it functions is not known. Expression of the human *bcl-2* gene in the nematode *Caenorhabditis elegans* reduced the number of programmed cell deaths, suggesting that the mechanism of programmed cell death controlled by *bcl-2* in humans is the same as that in nematodes.

Cell death is a normal part of development and homeostasis in both vertebrates and invertebrates (*1*). Extensive loss of neurons occurs during vertebrate development (*2*), and in mature animals cell death serves to eliminate autoreactive immune cells and virally infected cells (*3*). In mammals, *bcl-2* prevents some, but not all, cell deaths (*4–7*). For instance, expression of the *bcl-2* gene can prevent the death of myeloid or neuronal cells that occurs after withdrawal of certain growth factors (*4*), but it cannot protect cells from being killed by cytotoxic T cells (*7*). Inhibition of cell death by improperly regulated expression of *bcl-2* may also be oncogenic, as when *bcl-2* is activated by t(14;18) translocations in human follicular lymphoma (*8*) or when *bcl-2* is overexpressed in transgenic mice (*5*). The activation and execution of an intracellular suicide pathway is referred to as programmed cell death (*1*).

Programmed cell death occurs in the nematode *Caenorhabdits elegans*. Of the 1090 somatic cells formed in the course of the development of an adult worm, 131 cells undergo programmed cell death (*9*), and the genetic pathway by which cell death occurs has been extensively characterized (*1*). We tested whether programmed cell deaths in nematodes and humans can occur by way of the same molecular pathway by constructing a vector (*hsbcl-2*) that places expression of the human *bcl-2* gene under control of the nematode heat shock promoter (*10*) and generating transgenic nematodes with *rol-6* as a cotransformation marker (*11, 12*). Normally, cells undergoing programmed cell death are observable only for a short period, because the cell corpse is rapidly engulfed (*9*). We therefore used *ced-1(e1735)* mutants because this mutation prevents engulfment, allowing the cell corpses to accumulate (*13*).

We used antibody staining to demonstrate that transgenic nematode embryos express the human *bcl-2* protein. After heat shock, embryos from transgenic parents were fixed and labeled with monoclonal antibodies to human *bcl-2* (*14*), and then staining was visualized by fluorescence microscopy (*15*). Transgenic sequences in nematodes are carried and transmitted as an extrachromosomal array (*11*), so we expected to see *bcl-2* expression in those progeny that carried the transgene, and not in their nontransgenic siblings. A proportion of offspring from transgenic parents were positive for *bcl-2* staining (Fig. 1). Staining was most prominent in the perinuclear regions of the cytoplasm, remained strong for up to 8 hours after heat shock, and was still

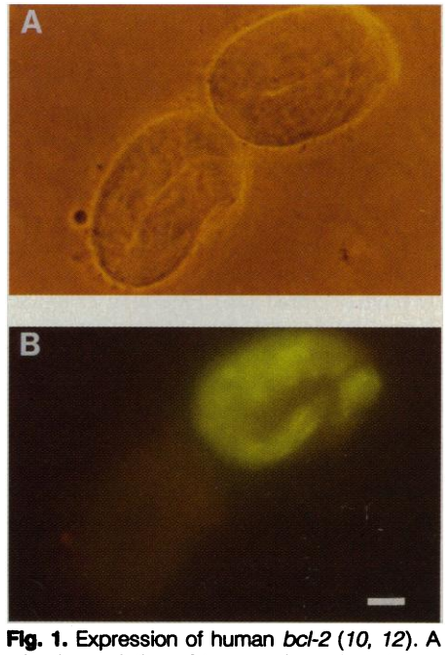

Fig. 1. Expression of human *bcl-2* (*10, 12*). A mixed population of transgenic and nontransgenic sibling embryos from *hsbcl-2* parents were stained with antibodies to human *bcl-2* and examined by phase contrast (**A**) and fluorescence microscopy (**B**) (*15*). Expression of *bcl-2* is seen in one of two embryos. Scale bar, 10 μm.

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