

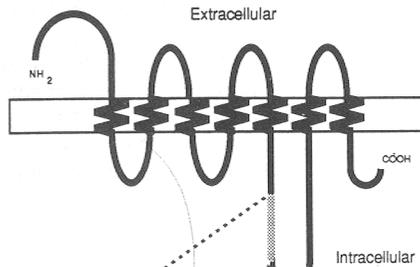
D₂ receptor, a missing exon

STR—A DNA sequence encoding a dopamine D₂ receptor in rat brain has recently been determined by Bunzow *et al.*¹. This sequence encodes a protein of 415 amino acids which is found in brain and anterior pituitary and is a member of a family of receptors which are coupled to G proteins. Interest in D₂ receptors stems

largely from their involvement in the pathology of neurological and psychiatric disorders such as parkinsonism², schizophrenia^{3,4} and drug addiction⁴.

Using a cloning strategy based on the polymerase chain reaction (PCR) and oligonucleotide primers corresponding to consensus sequences of the third and sixth transmembrane segments of this gene family⁵, we have obtained several clones from a rat pituitary complementary DNA library encoding the dopamine D₂ receptor. Sequence comparisons showed that these clones were consistent with the published structure except for an additional 29 amino acids in the third cytoplasmic loop inserted after Lysine 241 (see figure). Additional analysis of genomic DNA indicated that this insert is encoded by a separate exon between the flanking sequences reported by Bunzow *et al.*¹. This region of the receptor is involved in G-protein coupling and varies dramatically between different receptor subtypes⁶. There is also considerable evidence that the dopamine D₂ receptor can couple to either the cyclic AMP or the phosphoinositol second messenger pathways, or to both⁷. It is possible that the additional exon confers an alternative G-protein specificity on the D₂ subtype, through differential splicing of messenger RNA. Although receptors of this type are normally encoded by a single exon, the D₂

receptor is encoded by multiple exons¹. Using oligonucleotide primers corresponding to sequences upstream and downstream from this portion, we found by PCR that the larger form of the dopamine D₂ receptor predominates in both rat pituitary and brain cDNA. In neither tissue did we observe the smaller form.



Schematic diagram of the dopamine D₂ receptor showing the location of an additional exon in the third cytoplasmic loop. The amino-acid sequence of the exon is shown in single letter code above that of the previously published structure¹.

230 240 250 260 270 280
 RAFRANLKTPLKGNCTHPEDMKLCTVIMKSNNGSFPVNRMRDAARRAQQELE
 RAFRANLKTPLK-----DAARRAQQELE
 230 240 250

The northern analysis of RNA transcripts in brain and pituitary illustrated by Bunzow *et al.*¹ could therefore represent the larger or both forms of the receptor.

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Exxon Valdez bird toll

STR—On 24 March 1989, the oil tanker *Exxon Valdez* spilled 260,000 barrels of Alaska North Slope crude oil into Prince William Sound. Oil drifted in a south-westerly direction into the Gulf of Alaska and eventually covered 25,000 km² of coastal and offshore waters occupied by more than half a million marine birds. As predicted^{1,2}, we have witnessed an unprecedented toll of marine birds from oil pollution, which is summarized here.

Dead birds found on beaches and floating in open waters were retrieved by fishermen under contract to Exxon Oil Company, volunteers, and personnel from the US Fish and Wildlife Service (USFWS), Alaska Department of Fish and Game, International Bird Rescue

Center and other organizations. Oiled birds were processed and identified (when possible) by USFWS biologists. Data presented here (see Table) include birds retrieved between 25 March and 25 September, 1989.

Preliminary analysis of wildlife surveys conducted before^{2,3} and after (USFWS, unpublished data) the spill indicates that about 600,000 marine birds were present in areas contacted by oil. About half that number comprised species with high vulnerability to oil (such as guillemots). More than 35,000 dead birds (89 species) were retrieved from affected areas by 25 September 1989 (see Table). However, of 5,000 deaths — mainly of kittiwakes, puffins and shearwaters — in August and September, most are the result of natural causes. Most birds (90 per cent) were killed outside Prince William Sound in the Gulf of Alaska, and the relative composition of oiled birds varied markedly between those areas. In Prince William Sound, proportionally more coastal species were killed. In the Gulf of Alaska, common guillemots were most affected, with few other species comprising more than 2 per cent of the total kill. Species killed in large numbers relative to their local densities included yellow-billed loons, harlequin ducks, pigeon guillemots, marbled murrelets and bald eagles.

Based on the results of corpse-drift experiments conducted elsewhere^{4,6} and numbers of birds at risk, we tentatively conclude that the number of birds retrieved represents 10–30 per cent of the actual kill, which was probably between 100,000 and 300,000 birds. The lower estimate is conservative because outside Prince William Sound, logistics, weather and geography prevented a complete and timely search of affected areas. On the other hand, the upper estimate is probably high as it implies an almost total loss of populations at high risk — which we did not observe.

Few, if any, oil spills have had as large an impact on marine bird populations as the *Exxon Valdez* spill. Well-documented^{6–8} large oil spills, such as those from the *Torrey Canyon* (7,815 birds retrieved out of an estimated kill of 30,000), *Hamilton Trader* (4,092 out of 10,000), and *Amoco Cadiz* (4,572 out of 20,000), have rarely resulted in retrievals or estimated losses of more than 20,000 birds^{9,10}. A few lesser-known incidents of acute oil pollution in the North and Baltic seas have resulted in estimated losses of 30,000–50,000 birds, usually alcids and seaducks^{11–13}. On a global scale, the northern Gulf of Alaska harbours enormous populations of marine birds and so the magnitude of losses from the *Exxon Valdez* oil spill was predictable^{1,2}, and, not surprisingly¹, exceeds any other record of oil-related mortality we can find. If the spill had occurred in summer or autumn, the toll could have

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Total numbers and species composition of birds retrieved from affected areas after the Exxon Valdez oil spill

	Area		Total
	Prince William Sound	Gulf of Alaska	
No. retrieved	3,360	31,919	35,279
No. identified	2,884	29,468	32,352
Percentages:			
Loons and grebes	20.5	0.9	2.6
Procellariids*	0.5	14.4	13.2
Cormorants	16.0	1.3	2.6
Seaducks	24.8	2.3	4.3
Gulls	1.7	6.3	5.9
Guillemots	15.1	64.9	61.7
Other alcid†	17.2	9.7	9.1
Other birds	4.2	0.2	0.4

* Includes fulmars, shearwaters and storm-petrels.

† Includes pigeon guillemots, murrelets, auklets and puffins.

been much higher⁴. A few other locations in North America have the potential for similar bird losses, and many are the object of oil and gas exploration or development (for example, eastern Canadian Arctic, Grand Banks of Newfoundland).

Whether bird losses from the Exxon Valdez spill represent biologically significant losses in Alaska or will even be detectable in most populations remains to be seen. It will take years and even decades for some populations to return to pre-spill numbers^{14,15}, but other natural and artificial perturbations may obscure the effects of, or recovery from, oil mortality^{7,9,16,17}.

Bacterial zipper

SIR—An in-phase repetition of a leucine every seven residues in an α -helical structure is a motif associated with the dimerization of proteins and, together with an adjacent basic region, is responsible for the interaction of eukaryotic regulators and specific DNA sequences. This structural and functional motif has been termed the 'leucine zipper'^{1,2}. It is also present in membrane proteins that do not bind to DNA³⁻⁵.

In the course of our work on a new plasmid of *Pseudomonas savastanoi* (Nieto *et al.* in preparation) we have found that its replication protein (RepA) has a putative leucine-zipper motif in a sequence located at the N terminus. The computer

Comparison of N-terminal regions of four plasmid replication initiator proteins. Coordinates of initial and terminal residues are indicated. □, Leu repeats and other compatible residues; ○, other conserved positions. Average chemical character of residues: ●, non-polar; ○, polar; ⊕, positively charged. Underlined: helix-destabilizing residues. HDR: helix-destabilizing region.

Protein	Plasmid
RepA	pPS10
RepA	pSC101
E	F
π	R6K

Furthermore, even though we suspect that certain colonies were hard hit by oil, we were unable to identify where dead birds originated, and losses may therefore be spread over a larger geographical range than we surmise¹⁶. In any case, local populations may recover in 20–70 years, and the process will be accelerated if birds emigrate from unaffected colonies¹⁴⁻¹⁷.

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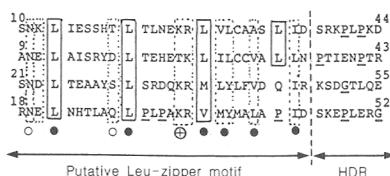
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predictions of secondary structure give this region a potential α -helical character. There are no residues incompatible with α -helix among the first four Leu residues but the two prolines that flank the fifth Leu residue should destabilize the α -helical structure; we therefore predict that the α -helix has about seven turns.

Comparison of this region of the RepA protein with known sequences of functionally equivalent proteins involved in initiation of replication in plasmids of Gram-negative bacteria, indicates that some of them⁶⁻⁸ also have a potential leucine zipper motif at the N₂-terminal region. The eventual absence of a leucine or a compatible residue at the fourth position and the presence of destabilizing residues could indicate, however, that all



Which Haldane?

SIR—Mark Williamson and Robert M. (Nature **341**, 695; 1989) in considering Haldane beetle story appear to err attributing it to J. B. S. Haldane, famous geneticist. Its origin is more likely to be his father, J. S. Haldane, the distinguished physiologist, who began his work in the physiology laboratory at Oxford in 1887, or possibly his uncle, R. B. Haldane who was Minister of War before becoming Lord Chancellor in 1912. In 1915 R. Haldane was excluded by Asquith from his coalition government because public opinion considered him pro-German because of his well-known enthusiasm for Kant and other German philosophers. This enthusiasm he had imparted to his young brother. Either J. S. or R. Haldane, as young men, might well have approached Jowett for advice on philosophy and been invited to dinner at his table at Balliol where the conversation now a legend, could have taken place.

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these sequences may represent a leucine zipper-like motif in prokaryotes in which shorter α -helices could also be functional.

Note that the reported initiator proteins share the properties of binding to DNA and of being transcriptional regulators of their own synthesis; it has been proposed that they also interact within themselves and with other proteins of the replication machinery of the cell^{9,10}.

A computer search for the DNA-binding helix-turn-helix motif¹¹ in RepA found a C-terminal region that could fit the structure. Similar observations have been reported for the other initiation proteins^{12,13}. The N-terminal leucine zipper-like motif described here may, therefore, be involved in protein-protein interaction.

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CONCEPCION NIETO

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