Elrathina zones; *Bolaspidella* zone; Dresbachian; Franconian; Trempealeauan; Gasconadan; Beekmantownian; Whiterockian; Chazyan; Blackriverian; Trentonian; Edenian; Maysvillian and Richmondian; lower and middle Llandoverian; upper Llandoverian; Wenlockian; Ludlovian and Pridolian; Lochkovian; Pragian; Emsian; Eifelian; Givetian; Frasnian; Famennian; Kinderhookian; Osagean; Meramecian; Chesterian; Morrowan and Atokan; Desmoinesian; Missourian; Virgilian; lower Wolfcampian; upper Wolfcampian; Leonardian; Wordian; and Capitanian and Ochoan.
8. No attempt was made to distinguish between true extinction (that is, lineage termination) and pseudoextinction (that is, phyletic transformation resulting in change of generic name).
9. Times of extinction were derived from a compilation of stratigraphic ranges of marine genera described in (*11*).
10. D. M. Raup and J. J. Sepkoski, Jr., *Science* **215**, 1501 (1982); L. Van Valen, *Nature (London)* **307**, 50 (1984); J. J. Sepkoski, Jr., in *Search for Extraterrestrial Life: Recent Developments*, M.D. Papagiannis, Ed. (Reidel, Dordrecht, 1985), p. 223.
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12. The large extinction intensities in environmental zone 6 reflect in part the inclusion of pelagic and nektonic taxa, including graptoloids and ammonoids, with very high characteristic turnover rates.
13. Friedman's test [S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956)] for five rows (Ordovician through Permian) and six columns yields $\chi_r^2 = 15.74$ ($P < 0.01$), indicating significant differences among the columns. Inclusion of the Cambrian, however, decreases χ_r^2 to 7.49, which is not significant ($0.10 < P < 0.20$). These and subsequent tests are only partially valid, however, since the rows and columns are not entirely independent (many genera occur in more than one environmental zone, more than one system, or both).
14. Friedman's test for the five rows (classes) and six columns (environmental zones) illustrated in Fig. 2 yields $\chi_r^2 = 11.40$ ($P < 0.05$).
15. These results suggest that possible adaptations to fluctuating environments, such as expanded niche breadth and greater larval dispersal, were not sufficient to counteract increased extinction intensities in the nearshore during the Paleozoic.
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17 June 1986; accepted 16 October 1986

Delayed Transneuronal Death of Substantia Nigra Neurons Prevented by γ-Aminobutyric Acid Agonist

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In an investigation of the mechanism by which brain lesions result in delayed degeneration of neurons remote from the site of injury, neurons within the caudate nucleus of rats were destroyed by local injection of the excitotoxin ibotenic acid. Treatment resulted in the rapid degeneration of the striatonigral pathway including projections containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and delayed transneuronal death of neurons in the substantia nigra pars reticulata. The distribution of nigral cell loss corresponded to the loss of GABAergic terminals. Neuronal death was prevented by long-term intraventricular infusion of the GABA agonist muscimol. Delayed transneuronal degeneration may be produced by neuronal disinhibition consequent to loss of inhibitory inputs. Replacement of inhibitory transmitters by suitable drugs may prevent some forms of delayed neuronal death.

WITHIN THE CENTRAL NERVOUS system (CNS), neurons may die when deprived of their afferent input (*1*). This form of neuronal degeneration, termed anterograde transsynaptic or transneuronal cell death (*1*), is believed to underlie some of the systemic degenerative diseases of the human nervous system (*2*). Its mechanism is unknown.

One example of anterograde transsynaptic degeneration is the death of neurons in the substantia nigra pars reticulata (SNr) after destruction by excitotoxins of neurons of the caudate nucleus (CN) (*3, 4*). Excitotoxin treatment results in death of the neurons in the CN (*5, 6*). Included are neurons projecting to the SN over the striatonigral tract, many of which contain the amino acid neurotransmitter γ-aminobutyric acid (GABA) (*7*). Because GABA is a potent and ubiquitous inhibitor of neuronal discharge (*8*) and because excessive excitation of neurons elicited, for example, by excitatory neurotoxins can lead to neuronal death (*9, 10*), the transneuronal death of SNr neurons produced by CN lesions may be due to loss of an inhibitory GABAergic input.

To test this hypothesis we have elicited transneuronal degeneration of neurons in the SNr by destroying CN neurons with the excitotoxin ibotenic acid (IBO) (*11*). We sought to establish (i) whether the neuronal loss is topographically related to the magnitude of the loss of GABAergic innervation and (ii) whether the death of neurons in SNr could be prevented by intraventricular administration of a highly specific agonist of GABA, muscimol (*12*).

Table 1. Effects of microinjection of ibotenic acid (IBO) into one caudate-putamen (CP) with or without intraventricular administration of muscimol on the neuronal and glial density in the CP.

Group (*n*)	Density (neurons per 10^6 μm^3)	
	Neuronal	Glial
Control (3)	78.0 ± 10.1	63.3 ± 7.9
Lesion (3)	2.3 ± 0.3*	292.3 ± 19.7*
Lesion + muscimol (3)	3.0 ± 0.2*	268.5 ± 14.7*

*Differs only from control ($P < 0.001$) (Newman-Keuls test of multiple comparisons). Cannulas were implanted into the lateral ventricle (*11*). Animals receiving muscimol were treated (10 ng/μl) for 15 days. On day 15 after IBO treatment, rats were killed by perfusion (*12*), and neuronal or glial density was assessed (*13*).

Male Sprague-Dawley rats were anesthetized with halothane. IBO was stereotaxically injected unilaterally into three sites (4 μg of IBO in 0.4 μl of saline at each site) in the CN. Control animals received an equal volume of saline. In some animals, in the same operation, a cannula was inserted into the lateral ventricle contralateral to the lesion, fixed to the skull, and sealed for subsequent intraventricular injections (*13*). Wounds were closed aseptically and the animals returned to their cages. The animals had no evidence of convulsive activity upon awakening.

At various times thereafter, the animals were reanesthetized and perfused transcardially with phosphate-buffered 4% paraformaldehyde. Brains were removed, sectioned, and stained with thionine or, in some instances, with antibodies to GABA to establish the distribution of the GABAergic innervation of SNr (*14*). We calculated the extent of the IBO lesion of the CN by measuring the area of neuronal loss on serial transverse sections of the striatum. To facilitate analysis, the SNr was divided into four regions at the level of the accessory optic nucleus (AON) (Fig. 1). These were designated as area 1, medial; area 2, dorsal central; area 3, ventral central; and area 4, lateral (Fig. 2). The dorsal and ventral central areas together were termed the central core. The extent of neuronal death in the SNr was assessed as a function of the reduced density of neurons (*15*).

In agreement with our earlier observation

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(*4*), local application with IBO destroyed more than 95% of CN neurons within 48 hours; over the next 15 days, a marked gliosis developed (Table 1). These lesions produce complete disappearance of anterograde but not retrograde transport from the CN and reduce by about 50% the biochemical markers of GABA throughout the entire substantia nigra (*16*). Neurons in the deep layers of the overlying cortex and small portions of the adjacent globus pallidus and nucleus accumbens showed variable damage.

By 20 days after the lesion was made, gliosis was marked, total volume was reduced, and about 20% of neurons within the SNr ipsilateral, but not contralateral, to the lesion were lost (Fig. 1, A and B, and Fig. 2). As previously reported (*4*), neuronal death, first apparent 5 to 7 days after the lesion was made, reached a maximum by 21 days. The loss of neurons in the SNr was not homogeneous. The greatest loss was concentrated in the central core (Fig. 2B), where 53 to 57% of the neurons died. A still substantial but lesser neuronal death (28%) was seen in the lateral subdivision, whereas cell loss in the medial subdivision (16%) did not significantly differ from that of the contralateral CN. Within the SNr the density of glial cells also increased substantially. In three animals glial cell density in the central core, after correction for shrinkage (*15*), was 86.2 ± 6.9, whereas 15 days after a CN lesion it was 184.9 ± 6.3 glial cells per 10^6 μm^3.

To assess whether the neuronal loss corresponded to the distribution of the GABAergic projections, we measured the distribution of GABA immunoreactivity within comparable zones of the SNr (Table 2). In controls, the SNr was diffusely and homogeneously stained with antibodies to GABA (Fig. 2A). After a lesion of the CN, GABA immunoreactivity was reduced throughout the SNr but most markedly in the central core (Fig. 2A). Relative quantitation of the decline of GABA immunoreactivity was done by quantitative immunocytochemistry (Table 2). GABA staining was greatly reduced in the dorsal central area (by about 82%), somewhat less reduced in the ventral central and lateral areas (between 61 and 68%), and least reduced in the medial subdivision (about 43%). Thus, the major GABAergic projection from the CN to SNr is topographically concentrated within the central areas of the nucleus, and the loss of GABA immunoreactivity corresponds to the magnitude of transneuronal cell degeneration.

We next sought to establish whether replacement of GABA by long-term infusion of the GABA agonist muscimol (*12*) would influence transneuronal cell death in the SNr. In the first experiments, we began intraventricular infusions of muscimol (10 ng/μl) within 1 to 2 hours after placement of an IBO lesion. The agent was continuously infused for 15 days with an osmotic pump (Alza). This treatment completely prevented the expected loss of neurons in the SNr (Fig. 1C). It influenced neither the loss of neurons nor gliosis produced in the CN by IBO (Table 1), nor did it modify gliosis or shrinkage of the entire SN (Figs. 1 and 2A).

We examined the effect of various doses of muscimol on the transneuronal cell death of the SNr neurons. Within 1 to 2 hours after placement of an IBO lesion, muscimol was infused (0.01, 0.1, 1, 10, or 100 ng/μl) for 15 days. On day 15 the rats were killed and the neurons in the central core of SNr counted. Muscimol over a range of concentrations from 0.1 to 10 ng/μl effectively prevented neuronal death (Fig. 3A) in the SNr. The lowest dose (0.01 ng/μl) of muscimol had no effect, but the highest dose (100 ng/μl) caused degeneration in the contralateral SNr, indicating that in high doses the agent not only failed to prevent neuronal degeneration but was itself toxic.

In another series of experiments we sought to determine whether it was necessary to maintain the infusion of muscimol for 15 days to prevent cell death. Four animals received a continuous infusion of muscimol (10 ng/μl) for only 5 days after IBO lesions of the CN. The animals were maintained for 10 days after termination of the infusion and then killed. Brains were sectioned and stained. When muscimol was infused for only the first 5 days after IBO lesions, the loss of SNr neurons was only partially prevented (Fig. 3B). Thus, the vulnerable period for the neurons either persists throughout the time of infusion or for a period only partially including day 5 after the lesion.

Our study suggests that the mechanism for the transneuronal degeneration of neu-

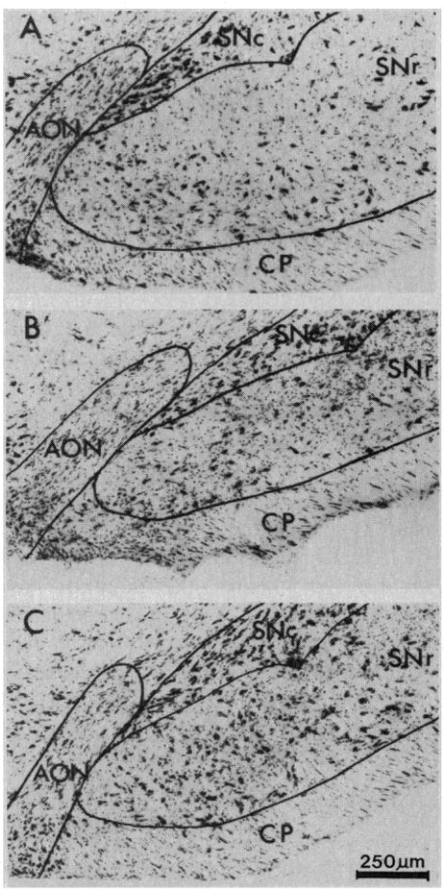

Fig. 1. Microphotographs of thionine-stained transverse sections of the SN at the level of the AON ipsilateral to the lesion, with or without long-term infusion of muscimol during 20 days after the IBO lesions of the CN. (**A**) Untreated control. (**B**) IBO lesion without muscimol. (**C**) IBO lesion with chronic infusion of muscimol (10 ng/μl, at an infusion rate of 0.5 μl per hour). Abbreviations: SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta; CP, cerebral peduncle; AON, accessory optic nucleus.

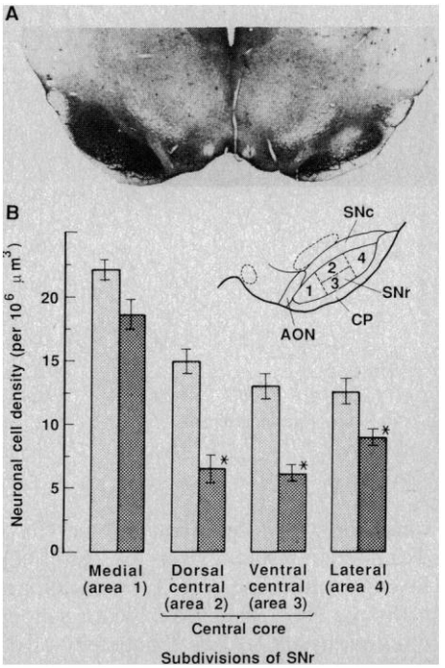

Fig. 2. (**A**) Transverse section of the SN immunocytochemically stained with antibodies to GABA (*14*) 15 days after unilateral lesions of CN by IBO. (**B**) Topographical distribution of the cell loss of neurons in SNr 15 days after the lesions of CN by IBO. Four areas of the SNr were defined as shown schematically in the insert: 1, medial; 2, dorsal central; 3, ventral central; 4, lateral areas. The cell density of neurons stained with thionine was measured (*15*) in each subarea of the SNr of unlesioned control rats (light bars), and rats lesioned with IBO (dark bars). Each bar represents data from four rats (means ± SEM); *, significantly different from unlesioned control (*t* test, $P < 0.05$). See the legend to Fig. 1 for abbreviations.

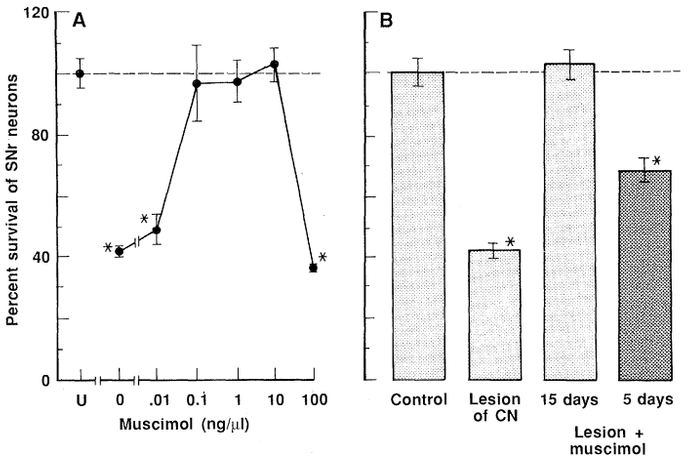

Fig. 3. (**A**) Effect of varying the dose of muscimol on transneuronal cell death of SNr neurons. Muscimol was infused for 15 days after the IBO lesions of the CN to groups of four rats at each dosage (mean ± SEM). U indicates the unlesioned control with saline injection. (**B**) Effect of period for infusion of muscimol (10 ng/μl) on the transneuronal cell death of SNr neurons. The treatment for each session was: control, unlesioned control with saline injection; lesion of CN, unilateral IBO lesion of CN; lesion plus muscimol for 15 days, unilateral IBO lesion with chronic infusion of muscimol for 15 days after lesion; lesion plus muscimol for 5 days, unilateral IBO lesion with chronic infusion of muscimol for only first 5 days after the lesion. The infusion rate was 0.5 μl per hour. Survival rate was estimated by measuring neuronal cell density (*13*). The values are means ± SEM for four rats per group; *, significantly different from all other groups ($P < 0.05$) by Newman-Keuls test of multiple comparisons. Cell densities [neurons per 10^6 μm^3 corrected for shrinkage (*13*)] are: control, 12.69 ± 0.16; lesion, 5.2 ± 0.44; lesion plus muscimol (15 days), 13.07 ± 0.66; and lesion plus muscimol (5 days), 8.9 ± 0.47.

rons of the SNr by lesions of the CN is disinhibition resulting from the loss of GABAergic projections of the striatonigral pathway. Three lines of evidence support this conclusion. (i) The topography of the uneven loss of SNr neurons corresponds exactly to the loss of the specific GABAergic projection of the striatonigral pathway. (ii) The transneuronal degeneration of SNr neurons was entirely blocked by the intraventricular administration of the specific GABA agonist muscimol (*10*). The response was dose-dependent and not evoked by injection of saline. Moreover, the muscimol effect was specific for the transneuronal degeneration since it blocked neither the toxic actions of IBO on CN neurons nor the degeneration of their processes within the SNr. (iii) Finally, iontophoretically applied GABA decreases the activity of SNr neurons (*17*).

Alternative interpretations, namely, that the delayed degeneration of SNr neurons may be a consequence of seizures initiated by the excitotoxin (*6, 10, 18, 19*) or that it results from diffusion of the toxin to the SNr, seem unlikely. Although repetitive generalized epileptic seizures elicited by inhalation of convulsants may delay degeneration of the SNr (*18*), such lesions are bilateral and are associated with glial as well as neuronal death, cytological features different from the results cited here. Moreover, although some excitotoxins, notably kainic acid, are highly epileptogenic, IBO is not (*19*). Nor is the CN a brain area in which seizures are easily elicited chemically or electrically. Finally, at no time after treatment did our animals exhibit behaviors that were compatible with seizure activity.

That the degeneration of SNr neurons resulted from a direct action of diffused IBO on SNr neurons also seems highly unlikely. Thus, the time course of direct neuronal degeneration produced by IBO or other neurotoxins (*6*) within the CN occurs within 24 to 48 hours, whereas the degeneration of SNr neurons after IBO treatment is delayed for about a week, with gradually increasing loss over the next 14 days (*4*). Moreover, the neuronal degeneration is partial and restricted to a topographically distinct area of the SNr and does not involve neurons of the immediately adjacent pars compacta of the SN. Finally, the diffusion distance from CN, 5 to 7 mm, is far greater than the zone of toxicity elicited by comparable amounts of IBO injected elsewhere in the brain (*20*).

Our study therefore demonstrates a new mechanism for transneuronal degeneration in the CNS: disinhibition by removal of specific inhibitory transmitters. These findings may be relevant to understanding certain kinds of delayed neuronal degeneration after head injury and stroke. Indeed, certain forms of ischemic-hypoglycemic mediated injury may be a consequence of the release of excitatory neurotransmitters from intrinsic pathways in the brain (*10*). Our findings suggest that withdrawal of inhibitory inputs may be another mechanism. Thus, GABA agonists might be used as possible therapeutic agents in preventing or attenuating the consequence of severe traumatic insults to the brain. In addition, selective loss of GABAergic neurons as, for example, within the striatum in Huntington's disease (*21*), may contribute to some of the secondary degeneration of other classes of intrinsic neurons within that nucleus, including the cholinergic ones. Finally, our study raises the question whether other inhibitory neurotransmitters such as glycine or some neuropeptides may also participate in the neuronally mediated transneuronal cell loss in the brain in certain pathological states.

Table 2. Distribution of arbitrary units of optical density (OD) of GABA-positive terminals in SNr stained immunocytochemically with antibodies to GABA (*11*) (mean ± SEM in four animals) at 15 days after unilateral IBO lesions of the CN.

Areas of SNr	OD*		
	Ipsilateral side (*I*)	Contralateral side (*C*)	$\frac{I}{C} \times 100$
	Lesioned		
Total area	0.12 ± 0.01	0.33 ± 0.06	37.1
Subarea			
Medial (area 1)	0.21 ± 0.01	0.37 ± 0.04	56.7
Dorso-central (area 2)	0.06 ± 0.01	0.33 ± 0.09	17.6
Ventro-central (area 3)	0.11 ± 0.02	0.34 ± 0.08	31.6
Lateral (area 4)	0.12 ± 0.02	0.31 ± 0.02	39.2
	Unlesioned		
Total area	0.31 ± 0.02	0.32 ± 0.02	96.9

*OD was corrected for shrinkage (*22*).

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13. The guide cannula was implanted in rats stereotaxically (coordinates: anterior, 8.0 mm; lateral, 1.4 mm; ventral, 1.5 mm). The guide cannula provided for subsequent placement of an internal cannula that protruded 3.0 mm beyond the end of the guide cannula into the lateral ventricle. Animals received long-term infusion (0.5 μl/hour) of muscimol by osmotic pump (Alza) connected to the internal cannula through plastic tubing.
14. Animals were perfused with 2% paraformaldehyde containing 0.4% acrolein. Sections were processed for the peroxidase-antiperoxidase method with antibodies to GABA (1:1000, Immuno Nuclear) [J. Storm-Mathison *et al.*, *Nature (London)* **301**, 517 (1983)].
15. We measured neuronal cell density (cells per 10^6 μm^3) at a magnification of ×500 by counting the number of neurons per unit square (200 by 200 μm^2) within the SNr of transverse section (30 μm thick) stained with thionine. Only stained neurons containing a clear nucleus were counted. To correct for regional differences, neuronal density was measured at 60 points within the SNr over five serial sections at the level of the AON of the midbrain and then averaged. Overestimation of cell density due to shrinkage of the SNr after lesions of the CN was corrected by determining the amount of shrinkage of SNr 15 days after the lesion. This was 0.68 for the animals lesioned with IBO, 0.73 for the animals lesioned with IBO plus infusion of muscimol, and 1.0 for the unlesioned control. To assess the size of the lesion of CN or the volumetric change of the SNr induced by IBO lesions of the CN, area measurement was performed under low magnification (×16 to ×25) with a computer-based image analysis system [R. H. Benno, L. W. Tucker, T. H. Joh, D. J. Reis, *Brain Res.* **246**, 225 (1982)].
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23. Supported by NIH grants HL18974 and NS03346.

10 April 1986; accepted 8 October 1986

Restoration of LDL Receptor Activity in Mutant Cells by Intercellular Junctional Communication

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Exchange of small molecules between cells through intercellular junctions is a widespread phenomenon implicated in many physiological and developmental processes. This type of intercellular communication can restore the activity of low-density lipoprotein (LDL) receptors in mammalian cells that are deficient in the enzyme UDP-Gal/UDP-GalNAc 4-epimerase. Pure cultures of the 4-epimerase mutant are unable to synthesize normal carbohydrate chains on LDL receptors and many other glycoproteins and therefore do not express LDL receptor activity. When these cells are cocultivated with cells expressing normal 4-epimerase activity, the structure and function of LDL receptors are restored to normal by the transfer of this enzyme's products through intercellular junctions. The formation of functional junctions does not require normal glycosylation of membrane proteins. Because many convenient assays and selections for LDL receptor activity are available, this mutant can provide a powerful new tool for biochemical and genetic studies of intercellular junctional communication.

CELLS IN CULTURE AND IN VIVO CAN exchange small molecules and ions through intercellular junctions, resulting in electrical and dye coupling and in metabolic cooperation (*1, 2*). This junctional communication has been implicated in the integration of tissue physiology (for example, cardiac muscle contraction) and in the regulation of growth and development (*3*). The study of the formation, structure, function, and regulation of communicating junctions, such as gap junctions, has depended in part on assays that are technically complex (for example, dye microinjection) or time-consuming (for example, autoradiography) and often hard to quantify. We have developed a new and simple approach for the biochemical and genetic analysis of intercellular communication. This approach is based on the induction of low-density lipoprotein (LDL) receptor activity in mutant Chinese hamster ovary (CHO) cells (clone ldlD-14) by intercellular communication.

Clone ldlD-14 is one of many LDL receptor-deficient mutants that cannot bind or internalize LDL (*4, 5*). These mutants define four genetic complementation groups, *ldlA, ldlB, ldlC*, and *ldlD* (*6*). The primary biochemical defect in ldlD-14 (an *ldlD* mutant) is a deficiency in the enzyme UDP-galactose/UDP-*N*-acetylgalactosamine (UDP-GalNAc) 4-epimerase (*7*). The epimerase deficiency prevents synthesis of UDP-galactose and UDP-GalNAc when ldlD-14 cells are grown in standard culture medium (with glucose as the sole sugar source). As a consequence, the ldlD-14 cells have general defects in the synthesis of galactose- and *N*-acetylgalactosamine (GalNAc)–containing glycoconjugates, including glycoproteins such as the LDL receptor. For example, *O*-linked oligosaccharides normally attached to serine and threonine side chains through GalNAc residues are not synthesized in *ldlD* cells. The *O*-linked oligosaccharide defect in *ldlD* cells dramatically decreases LDL receptor stability and function (*7*). The general glycosylation defects and the abnormalities of the LDL receptor's structure, stability, and function can be corrected by adding galactose and GalNAc to the culture medium (*7*). These sugars can be converted to UDP-galactose and UDP-GalNAc through salvage pathways that bypass the 4-epimerase defect.

The LDL receptor function in ldlD-14 cells can also be restored by cocultivating these cells with other types of cells, including wild-type and other classes of LDL receptor-deficient CHO cells and human diploid fibroblasts (*8*). Restoration depends on proximity, and cannot be mimicked by conditioned medium from inducing cells (*8*), suggesting that intercellular junctions may be required. To determine the effects of cocultivation on the structure of the LDL receptor, we metabolically labeled cells and examined the structure of immunoprecipitated receptors by gel electrophoresis (Fig. 1). The abnormal structure of the LDL receptor in ldlD-14 cells was corrected almost completely by cocultivation with ldlA-650, a receptor-negative mutant from the *ldlA* complementation group (*9*). As previously shown (*7*), the receptor structure was

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