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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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22. Overestimation of the optical density on the side of the SNr ipsilateral to the lesion due to a marked shrinkage of SNr was corrected by use of the rate of the shrinkage (0.68) at 15 days after the lesion against the contralateral side (1.0). The areas within the SNr were defined as shown in Fig. 1B.
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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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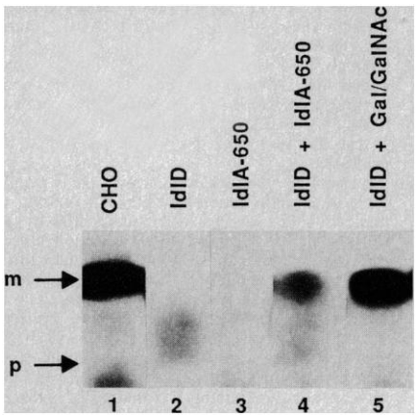

Fig. 1. Effect of cocultivation on LDL receptor structure in ldlD-14 cells. On day 0, 300,000 cells per 60-mm dish of the indicated pure cell types (lanes 1, 2, 3, and 5) or of a mixture of 80% ldlD-14 and 20% ldlA-650 cells (lane 4) were plated in 3 ml of medium A (Ham's F-12 medium supplemented with 100 U of penicillin and 100 μg of streptomycin per milliliter, 2 m*M* glutamine, and 3% newborn calf lipoprotein-deficient serum) (*23*). The pure cultures of ldlD-14 cells contained no additions (lane 2) or 10 μ*M* galactose and 100 μ*M* GalNAc (lane 5, additions also present in labeling and chase media). On day 2, the cells were labeled for 1 hour with 160 μCi of [^{35}S]methionine per milliliter, chased for 1 hour, harvested, and subjected to immunoprecipitation, electrophoresis, and autoradiography as described (*24*). A polyclonal rabbit antibody (anti-C) that recognizes the carboxyl terminus of the LDL receptor was used (*24*). Unless otherwise indicated, all experiments reported here were conducted at 37°C. The molecular weight standards "p" and "m" refer to the 125-kD precursor and the 155-kD mature forms of the LDL receptor (*24*). Cell types: CHO, wild-type CHO cells; ldlD, 4-epimerase–deficient and LDL receptor–deficient CHO cells (ldlD-14) from the *ldlD* complementation group; ldlA-650, LDL receptor–deficient CHO cells (defect in receptor's structural gene) (*9*).

also restored by incubation with galactose and GalNAc.

The restoration of essentially normal receptor structure and function in ldlD-14 cells by cocultivation could have occurred either as a result of the endogenous 4-epimerase activity being restored or as a result of the enzyme's products, UDP-galactose and UDP-GalNAc, being provided directly. Table 1 shows that cocultivation of ldlD-14 cells with wild-type CHO cells induced essentially normal LDL receptor activity in the ldlD-14 cells (activity in a 50:50 mixture was similar to that in 100% wild-type cells). In contrast, there was no effect on the 4-epimerase activity of ldlD-14 cells (activity in a 50:50 mixture was half of that in wild-type cells). Thus, the 4-epimerase defect in the ldlD-14 cells was bypassed, rather than corrected. This result could have been due to the transfer of UDP-galactose and UDP-GalNAc from the inducing cells to ldlD-14 cells through intercellular junctions. This possibility was examined by determining (i) the ability of ldlD-14 cells to form functional intercellular junctions and (ii) the relation between junctional communication and induction of receptor activity.

Two standard assays were used to assess the ability of ldlD-14 cells to form intercellular communicating junctions. First, dye [Lucifer yellow (MW 475)] microinjection and transfer experiments established that intercellular junctions formed between ldlD-14 cells themselves and between ldlD-14 cells and human diploid fibroblasts (*10*). This result was expected since others have shown that wild-type and mutant CHO cells readily form intercellular junctions (*11*). Second, an autoradiographic assay of metabolic cooperation (*2*) was used. To simplify this assay, we isolated a hypoxanthine-guanine phosphoribosyl transferase (HGPRT)–deficient variant of the ldlD-14 cells (ldlD-14h$^-$) (*12*). These HGPRT$^-$ ldlD-14h$^-$ cells could not directly incorporate [^{3}H]hypoxanthine from the culture medium into products insoluble in trichloroacetic acid (TCA) (Fig. 2); however, they could be labeled indirectly by the transfer of [^{3}H]hypoxanthine-derived metabolites from HGPRT$^+$ cells through intercellular junctions (*13*). Together, the microinjection and metabolic cooperation assays showed that small molecules can transfer through junctions between ldlD-14 and a variety of other cells.

To observe metabolic cooperation and LDL uptake simultaneously in individual cells, we incubated [^{3}H]hypoxanthine-treated cultures with fluorescently labeled LDL (Fig. 2) (*14*). For example, when ldlD-14h$^-$

Table 1. LDL receptor activity and 4-epimerase activity in pure and mixed cultures of wild-type and ldlD-14h$^-$ cells. LDL receptor activity was measured as follows. On day 0, the indicated cell mixtures (60,000 total cells per well) were plated into the wells of a 24-well dish in 1.5 ml of medium A, and on day 2, specific LDL receptor activity was measured with an ^{125}I-labeled LDL (10 μg of protein per milliliter, 310 cpm/ng) degradation assay (5 hours) (*8*). 4-Epimerase activity was measured as follows. On day 0, the indicated cells (480,000 in a 25-cm^2 flask) were plated in 12 ml of medium A. On day 2, the cells were harvested (one flask per point), and 4-epimerase activity in cytoplasmic extracts was measured at three different times per datum by means of a thin-layer chromatography assay (*7*).

Cell mix (%)		Activity	
CHO	ldlD-14h$^-$	LDL receptor* (ng/mg per 5 hours)	4-epimerase† (nmol/mg per hour)
100	0	2094	124
50	50	2422	63
0	100	13	0

*Values are the averages of duplicate determinations corrected for nonspecific degradation as described (*8*) and represent nanograms of ^{125}I-labeled LDL degraded per 5 hours per milligram of cell protein. †Values represent nanomoles of UDP-[^{14}C]GalNAc converted to UDP-[^{14}C]-labeled *N*-acetylglucosamine per hour per milligram of extract protein measured during the first 2 to 6 minutes of incubation. Analogous effects were observed with ldlD-14h$^-$ and CHO cells at ratios of 5:1 and 4:1.

Table 2. LDL receptor activity in monolayers of pure and mixed cells. LDL receptor activity was measured with an ^{125}I-labeled LDL (10 μg of protein per milliliter, 410 cpm/ng) degradation assay as described for Table 1, except that pure cultures of FH cells were plated at 25,000 cells per well, and mixtures of FH and ldlD-14h$^-$ cells contained 5,000 and 48,000 cells per well, respectively. See (*8*) for a detailed description of this assay and the effects of cell density, cell ratio, and culture conditions on induced receptor activity.

Cell type	LDL receptor activity (ng/mg per 5 hours)				Induction by cocultivation (%)‡
	Pure population, observed (a)	Mixtures containing 80% ldlD-14h$^-$			
		Calculated, no induction*† (b)	Observed (c)	Difference (c−b)	
CHO	2175	494	1678	1184	69
ldlA-7	17	63	1266	1203	70
FH†	164	82	1116	1034	53
LTA	1298	319	245	−74	−4
A431	5995	1258	1475	217	13
ldlD-14h$^-$					
No additions	74	74	74	0	0
+Gal/GalNAc‡	2220	74	2220	2146	100

*Column b represents the sums of 80% of the value for the ldlD-14h$^-$ cells alone (59.2 ng/mg per 5 hours) and 20% of the values in column a. †Values for FH-containing cell mixtures were calculated on the basis of cell ratios of 9.4% FH and 90.6% ldlD-14h$^-$ cells. Optimal induction of ldlD-14 cells occurs at ratios of 20:80 (*8*). ‡100% of maximal induction of the ldlD-14h$^-$ cells was the receptor activity in ldlD-14h$^-$ cells incubated for 2 days in medium B containing 10 μ*M* galactose and 100 μ*M* GalNAc and corrected to 80% of the total cells plated: 0.8 × (2220 − 74) = 1717 ng/mg per 5 hours. Fractional values (%) were calculated as (c − b) × 100/1717. For each cell type, similar results have been observed in at least two independent experiments (*8*).

cells were cocultivated with a receptor-negative *ldlA* mutant, ldlA-7 (*6*), LDL receptor activity (accumulation of fluorescent dye) was usually detected only in those ldlD-14h$^-$ cells that were in close proximity to an ldlA-7 cell and that were labeled with [^{3}H]hypoxanthine metabolites transferred from the ldlA-7 cell. In general, those ldlD-14h$^-$ cells that accumulated the most fluorescent LDL also contained the most transferred [^{3}H]hypoxanthine metabolites. However, there were radiolabeled ldlD-14h$^-$ cells that did not accumulate fluorescent LDL (*15*). The results were similar when LDL receptor–negative, HGPRT$^+$ human fibroblasts (FH cells) were cocultivated with ldlD-14h$^-$ cells (Fig. 2). Thus, for both FH and ldlA-7 cells, there was a strong but not absolute (*15*) relation between intercellular communication and induction of LDL receptor activity.

The possibility that induction of LDL receptor activity was due to junctional transfer of nucleotide sugars was also supported by studies with mouse LTA and human A431 cells. Both of these cell lines express functional LDL receptors (Table 2) and 4-epimerase activity. Mouse L cells (from which the LTA cells were derived) cannot form intercellular junctions with many different types of cells (*16, 17*). LTA cells did not transfer [^{3}H]hypoxanthine metabolites to ldlD-14h$^-$ cells and did not stimulate accumulation of fluorescent LDL (Fig. 2), suggesting that LTA cells cannot induce receptor activity in ldlD-14h$^-$ cells. This was confirmed by a quantitative assay of LDL receptor activity (Table 2). The A431 cells transferred [^{3}H]hypoxanthine metabolites to ldlD-14h$^-$ cells at a low but detectable level and induced a low level of receptor activity (Fig. 2 and Table 2) (*18*). In a control experiment, receptor activity in ldlD-14h$^-$ cells cocultivated with either LTA or A431 cells could be fully induced with galactose and GalNAc. Thus, cocultivation with these two cell types did not inhibit the intrinsic ability of ldlD-14h$^-$ cells to express LDL receptors. Together, these results suggest that LTA and A431 cells did not induce substantial LDL receptor activity in ldlD-14h$^-$ cells because they did not communicate efficiently with them.

The dependence of receptor induction on intercellular communication was also investigated with the use of retinoic acid, an inhibitor of junctional communication (*19*). Retinoic acid inhibited the induction of LDL receptor activity in ldlD-14h$^-$ cells by FH cells (Fig. 3A) and the transfer of [^{3}H]hypoxanthine metabolites (Fig. 3, B and C). However, retinoic acid did not block induction by galactose and GalNAc (Fig. 3A) and thus did not inhibit the

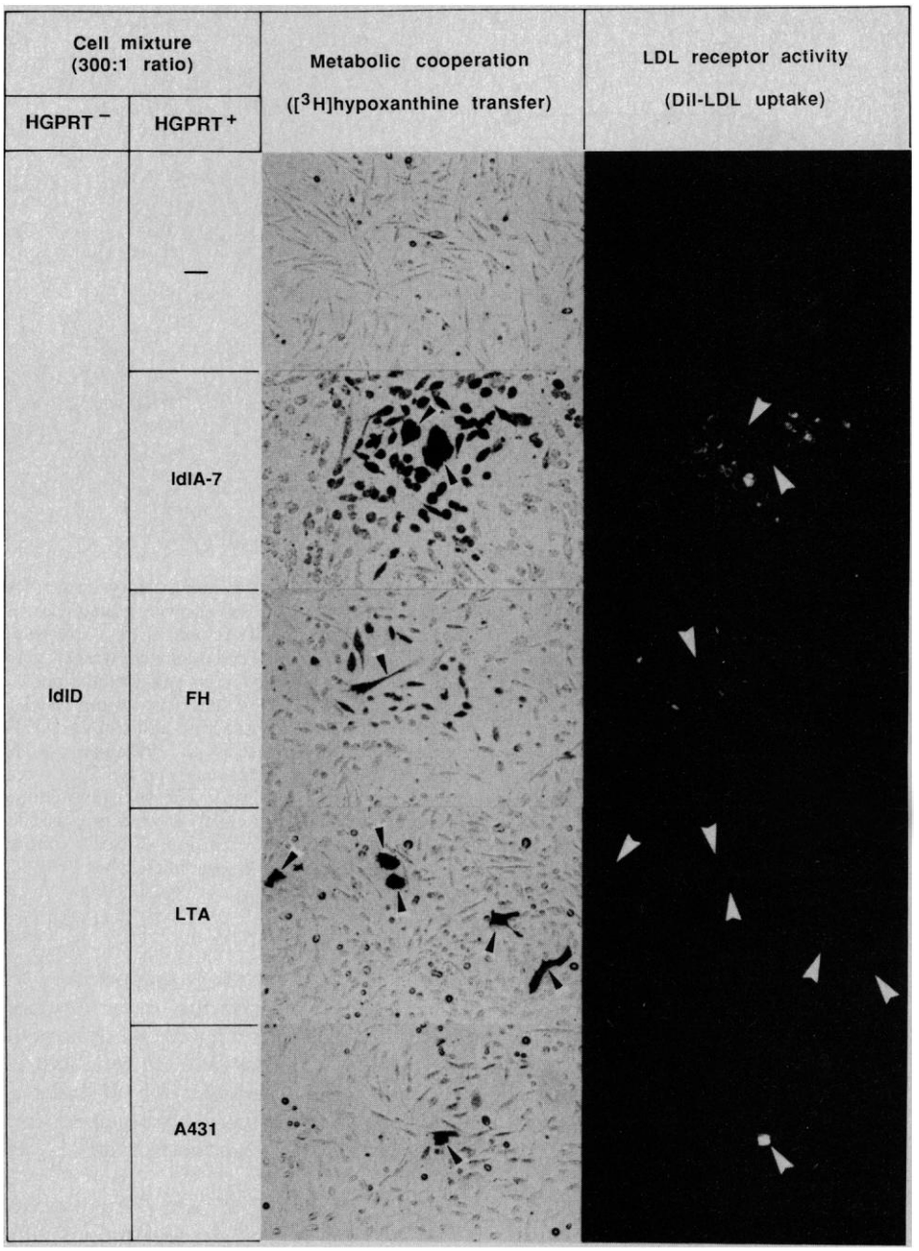

Fig. 2. Comparison of (**left**) metabolic cooperation and (**right**) induction of LDL receptor activity in ldlD-14h$^-$ cells. On day 0, HGPRT$^-$ ldlD-14h$^-$ cells (300,000 cells per 35-mm dish) were plated in the absence or presence of 1,000 cells per dish of the indicated HGPRT$^+$ cells (arrowheads) in 1.5 ml of medium A per dish prepared with hypoxanthine-free Ham's F-12 medium (KC Biologicals) and containing 2 μCi of [^{3}H]hypoxanthine monohydrochloride (10.0 Ci/mmol, New England Nuclear) per milliliter. On day 2, the cells were washed three times with complete Ham's F-12 medium and incubated for 7 to 9 hours with fluorescent LDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine-iodide–LDL (DiI-LDL) (10 μg of DiI-LDL protein per milliliter of medium A) (*6, 12*). Unincorporated [^{3}H]hypoxanthine was extracted with ice-cold 5% TCA (three times for 10 minutes each), and the dishes were washed overnight in water and air-dried at room temperature prior to autoradiography (Kodak NTB-2 emulsion, dried at room temperature and subsequently exposed at 4°C; total exposure time, 9 days). The ldlA-7, LTA, and A431 cells were identified by fluorescent microsphere labeling as described (*8*). The coumarin dye in the microspheres produces a fluorescein-like (λ_{em} = 540 nm) green fluorescence easily distinguishable from the rhodamine-like (λ_{em} = 631 to 640 nm) red fluorescence of the DiI-LDL; in this and previous work (*8*) there was no evidence of intercellular transfer of the microspheres themselves. In addition, the LTA, A431, and FH cells could be identified readily by their distinctive structures and their abilities to incorporate [^{3}H]hypoxanthine into acid-insoluble material. Matched bright-field (left) and fluorescence (right) micrographs (×10 objective lens) of representative fields were taken as described (*6, 8*). Cell types: ldlD, LDL receptor–deficient CHO cells (HGPRT$^-$ ldlD-14h$^-$); ldlA-7, LDL receptor–deficient CHO cells (defect in receptor's structural gene); FH, LDL receptor–deficient human fibroblasts derived from a patient with homozygous familial hypercholesterolemia (*8*); LTA, communication-defective mouse L cell line; A431, human carcinoma cell line. Three or more independent experiments were performed with each of these cell types, and comparable results were obtained in each case.

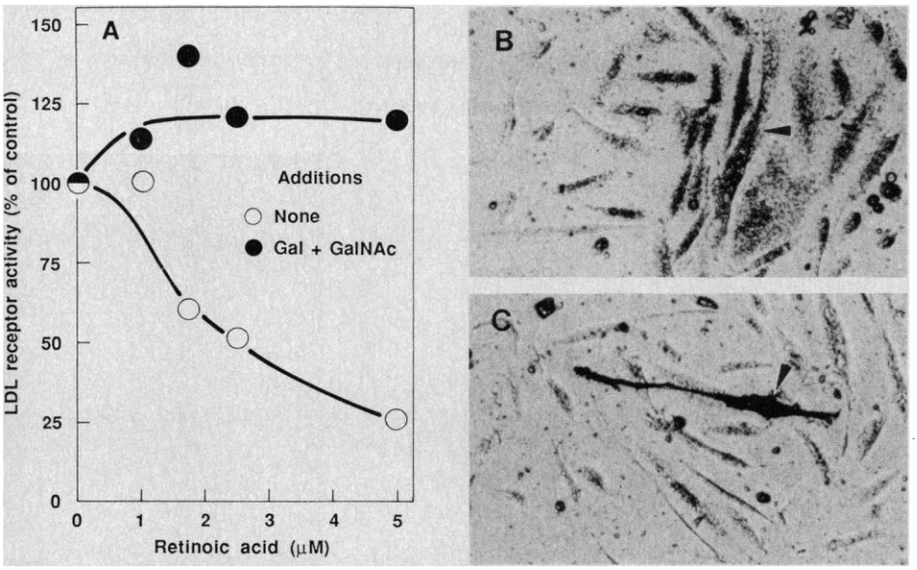

Fig. 3. Effects of retinoic acid on induction of LDL receptor activity and metabolic cooperation. (**A**) LDL receptor activity. On day 0, mixtures of 80% ldlD-14h$^-$ and 20% FH cells were plated with the indicated additions [retinoic acid (Sigma) added in dimethyl sulfoxide with (closed circles) or without (open circles) 10 μ*M* galactose and 100 μ*M* GalNAc]. On day 2, the monolayers were washed twice with Ham's F-12 medium, and then ^{125}I-labeled LDL (10 μg/ml, 351 cpm/ng) degradation was measured as described for Table 1 (no additions in the assay medium). The values representing 100% of control (per 5 hours) were as follows: no additions, 923 ng/mg; with galactose and GalNAc, 1573 ng/mg; the activities of pure cultures were as follows: FH, 16.5 ng/mg; ldlD-14h$^-$, 99 ng/mg. (**B** and **C**) Metabolic cooperation. Mixtures of ldlD-14h$^-$ (275,000 cells per 35-mm dish) and FH (1,000 cells per dish) cells were cocultivated for 2 days in the presence of 2 μCi of [^{3}H]hypoxanthine per milliliter and in the absence (B) or presence (C) of 5 μ*M* retinoic acid. The monolayers were prepared for autoradiography according to a slightly modified version of the procedure in Fig. 2 (phase contrast microscopy, ×20 objective lens). The FH cells previously labeled with fluorescent microspheres (Fig. 2) are indicated by arrowheads.

intrinsic ability of ldlD-14h$^-$ cells to express LDL receptors. Incorporation of [^{3}H]hypoxanthine by FH cells was unaffected by concentrations of retinoic acid (5 μ*M*) that blocked induction (Fig. 3C), which suggests that the inhibition of receptor induction was not due to nonspecific toxicity of retinoic acid. Furthermore, because retinoic acid did not interfere with normal glycosylation of LDL receptors in human fibroblasts, its effects on receptor induction cannot be attributed to inhibition of UDP-sugar synthesis in the FH cells.

Thus, experiments with an inhibitor of junctional communication and with cell lines having reduced ability to communicate confirm the correlation between intercellular communication and induction of receptor activity. Taken together, these data provide strong evidence that cocultivation-induced receptor activity in ldlD-14 cells was a consequence of the transfer of 4-epimerase–derived sugars through intercellular junctions (*20*). In addition, these data show that ldlD-14 cells could form functional junctions in the absence of 4-epimerase activity or the enzyme's products. Therefore, the synthesis of normal *N*-linked, *O*-linked, and lipid-linked oligosaccharides (*7*) was not required for junction formation and function. This conclusion is supported by our previous observation that receptor activity in ldlD-14 cells is induced by cocultivation with another glycosylation mutant, ldlB-11. This mutant is characterized by *N*-linked, *O*-linked, and lipid-linked abnormalities completely different from those of ldlD-14 cells (*8, 21*).

The dependence of LDL receptor activity in ldlD-14 cells on the formation of functional intercellular junctions provides an additional system for the biochemical and genetic analysis of intercellular communication. Fluorescence (*6, 8, 14*) and radioisotopic (*8, 22*) assays of LDL receptor activity are simple, fast, and easily quantified. For example, the LDL receptor assays require only 3 to 8 hours to perform after the cells have been cocultivated, whereas autoradiographic assays require weeks. In addition, many independent LDL receptor assays may be performed simultaneously, whereas dye transfer and electrical coupling assays require the penetration of many individual cells with microsyringes or microelectrodes. Therefore, the ldlD-14 cocultivation assay should prove useful for future studies. For example, inhibition of fluorescent LDL accumulation could be used to screen for specific antibodies or other compounds that block junction formation or function. Also, the availability of selections for and against the expression of LDL receptor activity in ldlD-14 cells may permit the isolation of mutants with defects in genes required for intercellular junctional communication (*5*).

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15. In some experiments, as many as 50% of the radiolabeled ldlD-14h$^-$ cells had not accumulated detectable amounts of fluorescence. These cells were usually farther from the inducing cells than ldlD-14h$^-$ cells that contained detectable amounts of both probes. The relative sensitivity of the autoradiographic assay depended in part on the exposure time (increased sensitivity with increased exposure time), whereas the relative sensitivity of the fluorescence assay depended on the concentration of fluorescent LDL, the amount of dye incorporated into the LDL, and the time of incubation with cells. Thus, very low levels of communication (for example, A431 and ldlD-14h$^-$ cells) were detected with greater confidence through the use of autoradiography and long exposure times than with the more rapid fluorescence or ^{125}I-labeled LDL degradation assays. In addition, differences in the time courses or levels of communication necessary for accumulation of detectable amounts of these two different probes may be responsible for these observations. For example, incorporation of [^{3}H]hypoxanthine-derived metabolites can normally be detected autoradiographically after relatively short periods of cocultivation (4 hours) (*17*), while the time to reach half of maximal induction of LDL receptor activity by cocultivation is about 14 hours (*8*).
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Decreased Hippocampal Inhibition and a Selective Loss of Interneurons in Experimental Epilepsy

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The occurrence of seizure activity in human temporal lobe epilepsy or status epilepticus is often associated with a characteristic pattern of cell loss in the hippocampus. An experimental model that replicates this pattern of damage in normal animals by electrical stimulation of the afferent pathway to the hippocampus was developed to study changes in structure and function that occur as a result of repetitive seizures. Hippocampal granule cell seizure activity caused a persistent loss of recurrent inhibition and irreversibly damaged adjacent interneurons. Immunocytochemical staining revealed unexpectedly that γ-aminobutyric acid (GABA)–containing neurons, thought to mediate inhibition in this region and predicted to be damaged by seizures, had survived. In contrast, there was a nearly complete loss of adjacent somatostatin-containing interneurons and mossy cells that may normally activate inhibitory neurons. These results suggest that the seizure-induced loss of a basket cell–activating system, rather than a loss of inhibitory basket cells themselves, may cause disinhibition and thereby play a role in the pathophysiology and pathology of the epileptic state.

IN HUMAN EPILEPSY, AN EPILEPTIC "focus," made up of an aggregate of abnormally discharging cells, develops as a result of physical injury or an unknown intrinsic mechanism ("idiopathic" epilepsy). Excitatory neural activity originating in the epileptic focus spreads to other brain regions, causing the behavioral and motor manifestations of the disorder. Seizure activity in the hippocampus, which occurs in temporal lobe epilepsy, the most common form of epilepsy, or status epilepticus, a condition of continuous seizure discharge, is often associated with a characteristic pattern of hippocampal damage. Irreversible cell loss is common in the hilus of the hippocampal area dentata and in the CA1 and CA3 pyramidal cell layers. The dentate granule and CA2 pyramidal cells are relatively resistant (*1*).

We developed an experimental epilepsy model (*2*) to study the functional and structural changes that occur as a result of seizure activity and may modify the course of the epileptic state or cause behavioral deficits. In this model, the pattern of hippocampal damage seen in the human epileptic brain (*1*) is induced in normal animals by electrical stimulation of the main excitatory pathway to the hippocampus (*2*). This experimental approach was designed to circumvent some of the interpretive problems inherent in previous studies (*3*) in which convulsant, and often directly neurotoxic, compounds are used to initiate seizures by usually unknown mechanisms. It is difficult to determine the role of seizure activity itself in seizure-associated brain damage when convulsant drugs are used since damage that might be caused by seizure activity cannot be readily distinguished from damage caused by direct neurotoxic actions of the drug. In addition, convulsants cause widespread seizure activity, motor convulsions, and widespread damage that precludes a comparison between damaged and undamaged sides of the same brain.

The model developed in this laboratory involves the induction of focal seizure activity with electrical stimulation of a single excitatory pathway. The mechanism by which seizure activity is induced is known, the seizures are evoked from an identified cell population directly innervated by the stimulated pathway and the seizure duration is controlled precisely. The resulting damage is restricted primarily to the stimulated hippocampus, thus permitting a comparison of structure and function in the stimulated and unstimulated hippocampi of the same brain. Since motor convulsions do not occur, the changes induced by seizures are not the result of metabolic effects associated with convulsions or a cessation of breathing. Furthermore, hippocampal granule cell seizure activity is recorded throughout the stimulation period, indicating that there is no lack of oxygenation in the hippocampus during the period when structure and function change. As a result, studies in this and other laboratories have demonstrated that it is the increased activity in excitatory hippocampal pathways that irreversibly damages cells (*2, 3*), probably by the release of excitatory amino acids in neurotoxic concentrations (*4*).

In recent studies of this model, we noted that the damage to dentate interneurons caused by granule cell seizure activity was associated with an acute decrease in granule cell recurrent inhibition (*2*). Inhibition is thought to be mediated by local interneurons that utilize the inhibitory amino acid γ-aminobutyric acid (GABA) as a neurotransmitter (*5*). It has been hypothesized that the loss of GABA-containing cells and the inhibition they produce shifts the excitatory-inhibitory balance, leading to seizure activity (*6*). Accordingly, we tentatively predicted

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