component ciliature, as well as the propagation of those anomalies under the direct influence of the existing ciliature. In *Pleurotricha*, most primordial fields develop without direct structural continuity with ciliature of a like kind. Indeed, when these hypotrichs encyst, all ciliature is broken down and an entirely new set is formed during excystment (*9*).

The mirror-imaged doublets reported here also are capable of encystment and excystment, and they do so true to type. Thus, redevelopment of the entire mirror-imaged pattern occurs without the direction of any existing ciliature, and illustrates that information for pattern asymmetry is retained in the cyst even in the absence of ciliature. If the overall pattern of the ciliature were the sum of individual events of the assembly of the ciliary components and there were no directive influence of existing ciliature, then the prediction would be that mirror-imaged doublets would not pass through the cyst true to type but rather revert to a typical symmetry. The fact that mirror-imagery is retained upon excystment further substantiates the conclusion that the mechanism of global patterning of the ciliature is independent of the mechanism of assembly of the individual ciliary components.

Although Tchang Tso-run and coworkers (*10*) have studied this type of mirror-imaged doublet and we have studied a different type of mirror-imaged doublet (*11*), none of those studies presented data on the internal organization of the ciliary components. Presumably, this is why the conclusions that we present have not been reached previously.

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5. The details of structure, standard development, and regenerative morphogenesis of *Pleurotricha lanceolata* have been studied (M. E. McKenna and G. W. Grimes, in preparation).
6. Mirror-imaged doublets were obtained either by heat-shocking (41°C; 17 minutes) random cultures of cells in the log phase of growth and subcloning or by subcloning surgically induced longitudinal fragments of cells in the log phase of growth. The exact mechanism of mirror-image induction remains to be elucidated.
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Prolongation of Islet Xenograft Survival Without Continuous Immunosuppression

Abstract. *The survival of isolated rat islets transplanted into diabetic mice was prolonged markedly by maintaining the rat islets in vitro at 24°C for 7 days before transplantation and administering to the recipients a single injection of antiserum to mouse and rat lymphocytes shortly before transplantation.*

Prolonged survival of thyroid allografts was obtained by Lafferty *et al.* (*1*) when they cultured donor thyroids in vitro for several weeks before transplantation. The thyroids were maintained in an atmosphere of 95 percent O_2 during the culture period. The cultured thyroids were rejected if donor peritoneal exudate cells were injected into the recipients at the time of transplantation (*2*). The authors suggested that passenger leukocytes in the thyroid had been destroyed by the culture conditions and that these lymphoid cells were required to initiate rejection of the thyroid. The method of organ culture used by Lafferty *et al.* (*1*) has also produced prolonged survival of thyroid xenografts (rat to mouse) (*3*).

Recently, we reported that islet allografts survived for prolonged periods (> 100 days) across a major histocompatibility barrier in rats when the islets were cultured in vitro at 24°C for 7 days before transplantation and the recipients were given a single injection of rabbit antiserum to rat lymphocytes (RALS) shortly before transplantation (*4*). Low-temperature culture of the islets for 1 to 4 weeks without the single injection of RALS did not produce a marked prolongation of allograft survival. Injection of donor peritoneal exudate cells produced prompt rejection of successfully established islet allografts in histoincompatible hosts at 100 to 220 days after islet transplantation. Since culture of the islets in vitro at low temperature and a single injection of RALS were effective in prolonging islet allograft survival, we sought to determine whether this simple procedure could be used to prolong the survival of rat islets transplanted into diabetic mice. Previous studies had shown that transplants of rat islets induced normoglycemia in diabetic mice for 1 to 5 days before rejection (*5*) and that normoglycemia could be maintained for 9 to 21 days by continuous immunosuppression of the recipients with antiserum to lymphocytes (*6*).

Male BALB/c mice were made diabetic by the intravenous injection of streptozocin (220 mg per kilogram of body weight). Concentration of plasma glucose was determined in the nonfasting mouse on blood obtained from the orbital sinus; only mice with plasma glucose concentrations > 400 mg/100 ml were used as recipients. Plasma glucose concentrations were determined three times weekly before and after transplantation, and the animals were weighed daily. The transplant was considered to be rejected when the plasma glucose level of the nonfasting animal exceeded 200 mg/100 ml.

Islets were isolated from male Wistar Furth rats by the collagenase technique (*7*) and separated on a Ficoll gradient (*8*). Islet tissue was removed from the gradient, and with the aid of a dissecting microscope, islets free from attached vascular and ductal tissue were selected and removed with a Pasteur pipette. The selected islets were then examined again under a dissecting microscope with a reflected green light for illumination, since this procedure permitted the identification and removal of small lymph nodes that are occasionally present in the preparation (*9*). The isolated rat islets were maintained in vitro in tissue culture medium (CMRL-1066) containing fetal calf serum (10 percent), penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and D-glucose (1.5 mg/ml). The islets were incubated in untreated plastic culture dishes and were maintained in an atmosphere of air and 5 percent CO_2 at 24°C for 7 days.

We found that the portal vein technique (*10*) could be used for transplanting islets into diabetic mice. Isografts of 650 BALB/c islets transplanted via the portal vein produced normoglycemia in diabetic recipients within 2 to 4 days. Five isografts (BALB/c) were still maintaining normoglycemia in the recipients 125 to 150 days after transplantation. Rat islets are slightly larger than mouse islets, and we found that transplants of 450 rat islets produced normoglycemia in

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Table 1. Effect of culture of islets in vitro and injection of antiserum to lymphocytes on survival of islet xenografts (rat to mouse).

Group	Transplant survival* (days per individual)	Percentage surviving days after transplantation					
		10	20	30	40	50	60
1. Control, fresh islets	7, 7, 7, 8, 8, 9, 9, 9, 10, 11, 12	27	0	0	0	0	0
2. Fresh islets (MALS, day 0)	15, 18, 20, 22, 25, 27, 34, 36, > 70, > 75, > 82	100	73	45	27	27	27
3. Cultured islets (24°C) (MALS, day 0)	21, 24, 24, 27, 31, 31, > 62, > 65, > 75, > 85	100	100	60	40	40	40
4. Culture islets (24°C) (MALS + RALS, day 0)	32, 41, 50, > 62, > 62, > 65, > 77, > 85, > 85, > 116	100	100	100	90	70	70

*The transplant was considered to have been rejected when the plasma glucose concentration (nonfasting) exceeded 200 mg/100 ml.

diabetic mice within 2 to 4 days. Thus we used 450 freshly isolated or cultured rat islets for transplantation into each diabetic mouse recipient. Rabbit antiserums to mouse lymphocytes (MALS) and RALS were used, and either MALS (0.2 ml) alone or MALS (0.2 ml) and RALS (0.1 ml) were injected intravenously into the diabetic mice 5 to 10 minutes before transplantation of rat islets. There were four transplant groups. Two groups received transplants of freshly isolated rat islets: group 1, without MALS; group 2, with a single injection of MALS. The other two groups received transplants of rat islets that had been cultured for 1 week at 24°C: group 3, with one injection of MALS; group 4, with one injection of MALS and RALS.

Transplants of freshly isolated rat islets were rejected between 7 and 12 days, with a mean survival time of 8.8 ± 0.5 days (group 1, Table 1). An indication of the pattern and rate of complete rejection of the islets could be obtained by examining the changes in the plasma glucose concentrations after transplantation (Fig. 1). In the control group, rejection was apparently beginning after day 6 and continued in a rapid linear fashion to day 15, when the plasma glucose concentrations had returned to pretransplant levels (Fig. 1A). In group 2, a single intravenous injection of MALS before transplantion of freshly isolated rat islets increased the survival time to 15 to 36 days in eight mice, and three other mice were still normoglycemic at 70, 75, and 82 days after transplantation (Table 1). Although the onset of rejection was delayed, the rate and pattern for complete rejection were similar to those of group 1 (Fig. 1B). The use of islets cultured in vitro at 24°C for 7 days, in conjunction with a single injection of MALS immediately before transplantation (group 3), produced a slight increase in transplant survival at 60 days after transplantation (40 percent) as compared to the 27 percent survival in group 2 (Table 1). The onset of rejection was also slightly delayed as compared to group 2 (Fig. 1, B and C). The most dramatic effect on islet xenograft survival was obtained with transplantation of rat islets cultured in vitro at 24°C for 1 week, together with a single injection of both MALS and RALS administered shortly before transplantation (group 4). In seven of this group of ten recipients, the islet xenografts were surviving at 60 days after transplantation, and these seven mice were still normoglycemic 62 to 116 days after transplantation (Table 1). We shall continue to monitor these seven animals to determine whether indefinite survival of the islet xenografts has been produced by this procedure. In three of the recipients in group 4, the islet xenografts were rejected at 32, 41, and 50 days after transplantation. The pattern for complete rejection of the islets in two of these animals was very rapid and resembled the rate of rejection observed in the control group (Fig. 1, A and D). The rate of complete rejection in the third animal was much slower, requiring approximately 28 days to approach this recipient's pretransplant plasma glucose con-

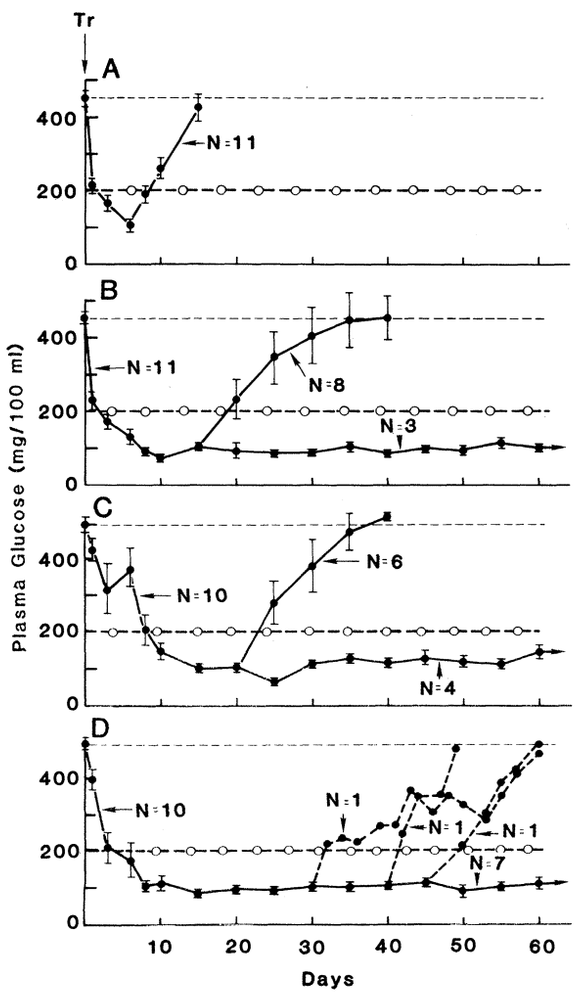

Fig. 1. Glucose concentrations in plasma of nonfasting diabetic mice (BALB/c) after they received transplants of isolated rat islets (Wistar Furth). (A) Group 1, transplants of freshly isolated rat islets. (B) Group 2, transplants of freshly isolated rat islets and one injection of MALS (0.2 ml) before transplantation. (C) Group 3, transplants of rat islets after in vitro culture (24°C) for 7 days and one injection of MALS (0.2 ml) before transplantation. (D) Group 4, transplants of rat islets after culture in vitro (24°C) for 7 days and one injection of MALS (0.2 ml) and RALS (0.1 ml) before transplantation. In each panel, the lower horizontal line (O) represents rejection when the plasma glucose exceeds 200 mg/100 ml, and the upper horizontal line (- - -) represents the mean pretransplant levels of plasma glucose for each group. The vertical bars indicate the standard error of the mean.

centration of 519 mg/100 ml. The role of the single injection of RALS in producing such a marked effect on preventing rejection of the xenografts of rat islets is unknown. The theoretical basis for including RALS in the experimental protocol was that the antiserum might destroy passenger leukocytes that were still present in the rat islets at the time of transplantation.

These findings indicate that a marked prolongation of survival of transplants of rat islets and maintenance of normoglycemia in diabetic mice can be achieved by culture of the rat islets in vitro for 7 days at 24°C prior to transplantation and by administration of a single injection of MALS and RALS into the recipients at the time of transplantation. Eloy *et al.* (*11*) reported recently that transplants of 15-day-old chick pancreases into diabetic rats improved the diabetic state of the recipients for a prolonged period of time whereas transplants of 18-day-old chick pancreas did not produce prolonged improvement. The development of methods for the prevention of immune rejection of islet xenografts without the use of continued immunosuppression may be of assistance in the eventual application of islet transplantation to the treatment of diabetes in man, since the source of islet tissue for human transplants may be a critically limiting factor.

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Assignment of the Murine Interferon Sensitivity and Cytoplasmic Superoxide Dismutase Genes to Chromosome 16

Abstract. *Both hybrids of mouse and human microcells and whole cell hybrids generated by the fusion of primary mouse cells and SV40-transformed human fibroblasts were used to establish the syntenic association of the murine cytoplasmic superoxide dismutase and the interferon sensitivity genes on mouse chromosome 16. This assignment adds two new markers to chromosome 16 and provides another example of an evolutionarily conserved linkage. This finding also provides an animal model both for cellular responsiveness to interferon and for Down's syndrome.*

Murine genes have been assigned to linkage groups and specific chromosomes by a combination of breeding experiments and somatic cell genetic studies. Hybrids of Chinese hamster cells with mouse somatic cells have generally been used in mouse mapping studies, since mouse chromosomes are often segregated (*1*). For certain genetic markers, however, the mouse and Chinese hamster gene products cannot be distinguished from each other, making primate × mouse hybrids desirable for murine gene mapping.

Table 1. SOD-1: assignment to chromosome 16. To ascertain the gene assignment, three different statistical procedures were used, χ^2, ϕ, and OR. The χ^2, used as a method of dependence, rather than a test of significance, was calculated in the ordinary way. The ϕ coefficient, which is insensitive to sample size, is the ratio of the observed χ^2 to the perfect χ^2. It is calculated by dividing the square root of the regular χ^2 by the square root of the sample size. When there exists a perfect association between a marker and a chromosome, the value of ϕ is +1. Zero indicates no association; −1 indicates a negative association. The OR statistic was designed for determining gene assignments (*19*). It is an inverse weighted scoring system which is independent of χ^2. It is calculated by assigning each clone a score of 100 and dividing that value evenly among all chromosomes in concordance with the tested marker. The clones are scored, the scores are added for each chromosome, and then divided by the number of clones. The chromosome with the highest number is considered a candidate for assignment of the gene for SOD-1. Generally an OR score of 7 or above is considered evidence for a positive assignment. Statistical analysis was conducted with computer program ASSIGN, developed for the purpose of gene assignment with material generated by somatic cell hybridization (*19*).

Chromosome	SOD-1/chromosome (number of clones)*				ϕ	χ^2	Overall OR
	+/+	+/−	−/+	−/−			
1	3	5	3	12	0.19	0.83	3.95
2	3	5	2	13	0.28	1.79	3.51
3	7	1	9	6	0.28	1.86	5.11
4	6	2	8	7	0.21	1.03	4.24
5	4	4	3	12	0.31	2.22	3.93
6	2	6	2	13	0.15	0.49	3.23
7	2	6	1	14	0.26	1.55	3.49
8	4	4	4	11	0.23	1.25	3.88
9	3	5	5	10	0.04	0.04	2.71
10	3	5	4	11	0.11	0.29	3.01
11	3	5	3	12	0.19	0.83	3.24
12	5	3	11	4	−0.11	0.29	2.92
13	4	4	1	14	0.50	5.76	4.21
14	3	5	1	14	0.39	3.45	3.72
15	5	3	8	7	0.09	0.18	3.44
16	8	0	2†	13	0.83	15.95	7.90
17	6	2	4	11	0.46	4.96	5.55
18	6	2	8	7	0.21	1.03	4.54
19	5	3	10	5	−0.04	0.04	3.63
X	1	7	4	11	−0.16	0.62	2.56

*For chromosomes, + indicates the presence of at least one copy in more than 20 percent of the cells analyzed. The sensitivity of the starch-gel electrophoresis assay for human SOD-1 is relatively low, as determined by dilution assay (*18*). The electrophoretic assay for murine SOD-1 is similarly insensitive. The following isozymes were analyzed in confirmation of chromosome identification: dipeptidase-1 (E.C. 3.4.—) and isocitrate dehydrogenase (E.C. 1.1.1.42) for chromosome 1; enolase (E.C. 4.2.1.2) for chromosome 4; β-glucuronidase (E.C. 3.2.1.31) for chromosome 5; triosephosphate isomerase (E.C. 5.3.1.1) for chromosome 6; lactate dehydrogenase A (E.C. 1.1.1.27) and glucosephosphate isomerase (E.C. 5.3.1.9) for chromosome 7; adenine phosphoribosyltransferase (E.C. 2.4.2.7) for chromosome 8; mannosephosphate isomerase (E.C. 5.3.1.8) and malate dehydrogenase (E.C. 1.1.1.40) for chromosome 9; tripeptidase 1 (E.C. 3.4.—) for chromosome 10; galactokinase (E.C. 2.7.1.6) for chromosome 11; purine nucleoside phosphorylase (E.C. 2.4.2.1) and esterase 10 for chromosome 14; dipeptidase 2 for chromosome 18; glutamate oxaloacetate transaminase (E.C. 2.6.1.1) for chromosome 19; phosphoglycerate kinase (E.C. 2.7.2.3) and glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) for chromosome X. The method of starch-gel electrophoresis of isozymes including cytosol SOD-1 has been described (*4*, *18*). Since our hybrid cells segregate mouse chromosomes rapidly, chromosome spreads and harvesting of cell pellets for isozyme analysis were generally performed within a week of each other. †These two lines (SxO_2-1 and SxA_2-1) have chromosome frequencies of 26 and 21 percent which are the limit (about 20 percent) for detection for SOD by starch-gel electrophoresis.

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