missing. In summary, starting with the original drug-selected progeny of the haploid cross, the micronuclear chromosome set was taken from double monosomic to single nullisomic to single monosomic, and finally to single nullisomic. Thus the separation of germinal and somatic function into two different nuclei allows us to experimentally generate unique genotypes in the germ line which would be lethal in a nucleus that had somatic function.

A first use of nullisomics has been to identify the chromosome location of as many genetic markers as possible. When a strain homozygous for a mutation is crossed with a wild-type diploid, the progeny are heterozygous. In *Tetrahymena* this means the culture originally expresses the dominant phenotype, but subcultures expressing the recessive phenotype can be established following phenotypic assortment. If the mutant strain is crossed to a nullisomic strain, the progeny will be either heterozygous if the marker is not on the chromosome missing in the nullisomic strain or hemizygous if it is on that chromosome. A hemizygous culture should stably express the mutant phenotype, with no wild-type cells occurring by dominance or assortment. Thus it does not matter whether a mutation is dominant or recessive; a hemizygous culture will give a phenotype different from a heterozygous one.

Together with collaborating colleagues (*12*), we have thus far crossed our nullisomics with about 60 strains homozygous for known mutations. Four morphological mutations (*cdaC, D, H,* and *fatD*) and two cell-surface antigen control loci (*r3, R5*) have been found to be on chromosome 5 (*13*). We are also using the nullisomics to isolate new mutations on designated chromosomes (*14*).

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Diameter of the Cell-to-Cell Junctional Membrane Channels as Probed with Neutral Molecules

Abstract. *The cell-to-cell channels in the junctions of an insect salivary gland and of insect and mammalian cells in culture were probed with fluorescent molecules—neutral linear oligosaccharides, neutral branched glycopeptides, and charged linear peptides. From the molecular dimensions of the largest permeants and smallest impermeants the permeation-limiting channel diameter was obtained: 16 to 20 angstroms for the mammalian cells and 20 to 30 angstroms for the insect cells.*

The cell junctions in many organized tissues contain membrane channels directly linking the cell interiors with each other (*1*). Probings with a series of hydrophilic, linear peptide molecules showed that these cell-to-cell channels are at least 16 Å in diameter (*2, 3*)—the abaxial dimension (width) of the largest permeant molecule in this series—and that they can select against negatively charged, large permeants (*3, 4*). The probing molecules were charged and, therefore, did not allow a distinction between steric and polar channel constraints; the actual channel diameter could be larger. In particular, the channels in insect cell junctions could be larger; they were less permselective than those in mammalian cells (*3*). In the work reported here we probed the channels of mammalian and insect cells with neutral sugar molecules to assess their diameter more closely.

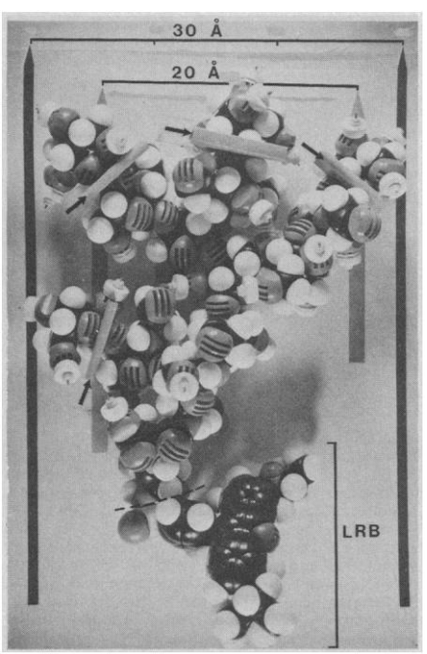

Fig. 1. Space-filling model of the branched glycopeptide tracer molecules. Shown is the LRB-labeled glycopeptide. The four terminal galactoses of this molecule are missing in the agalactopeptide; the planes (arrows) indicate where the galactoses are clipped off in the preparation of this molecule. The widths, the primary permeation-limiting dimensions of the two molecules, are given; other dimensions are to scale. The molecules weigh 3097 and 2449 daltons. For the chemical structure, see footnote in Table 1. [Another set of these molecules is labeled with FITC (2975 and 2327 daltons) or RB (3014 daltons); their widths are the same.]

The probes, a series of fluorescent, linear oligosaccharides and a set of fluorescent, branched glycopeptides, are listed in Table 1. The sugar units of the linear oligosaccharide chains were either α- or β-linked and, in the case of the dimers, the two chains were linked by the fluorophore. The oligosaccharides were derived from starch by partial acid hydrolysis (*5*) or from cellulose by acetolysis (*6*). A primary or secondary amino group was introduced into these molecules by reductive amination (*7*), and the fluorophore fluorescein isothiocyanate (FITC) was covalently linked to it as fluorescein thiourea (FTU). The glycopeptides were obtained by exhaustive proteolysis of desialized α_1-acid glycoprotein (orosomucoid) from human plasma (*8*). Additional treatment with β-D-galactosidase produced the agalacto member of this set. They were labeled at the threonyl-residue amino groups with FTU, rhodamine B (RB), or lissamine

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rhodamine B (LRB). All of the compounds were purified in several steps by cation exchange chromatography, gel permeation chromatography, and thin-layer chromatography. They showed no sign of permeating nonjunctional cell membrane from the outside (*9*) or of being degraded by the cytoplasm (*10*).

The linear molecules were used in the salivary gland of the insect *Chironomus thummi* (mid-fourth instar) and the branched ones in this organ and in cultures of the insect cell line AC-20 and of the mammalian cell lines B, RL, and 3T3-BALB/c (*11*). Monoglutamate and diglutamate labeled with LRB [LRB(Glu)OH and LRB(Glu)$_2$OH, respectively] were used as accessory probes in the mammalian cells (*12*). The probes were microinjected into the cells and their cell-to-cell diffusion was observed in a dark-field microscope (*13*). Observation periods were up to 10 minutes. The injected cells were multijunctional. Any given probe was found to pass, when it did at all, to various cell neighbors in all injection trials: to first- and second-order neighbors (and often even to higher order ones) in the gland (except for the α-linked dimers, which generally passed detectably only to the first-order neighbors) and to at least 80 percent of the first-order neighbors in the cultures. Each injection trial thus served to test several junctions at a time.

The results are summarized in Table 1. All of the linear oligosaccharides passed through the gland cell junction; the monomers and β-linked dimers were the most permeant. As given by space-filling models, the width of the largest molecules in this series—their primary permeation-limiting dimension—is 16 to 20 Å, depending on the conformation of the dimers about the fluorophore linkage. Accordingly, the channels would have to be at least this wide.

The most revealing results were obtained with the set of branched glycopeptides. This set consists of a molecule with four terminal galactoses and a second molecule, that same glycopeptide, but with the galactoses clipped off. Figure 1 shows the complete molecule and Table 1 (footnote #) gives its primary chemical structure. The permeation-limiting widths of these molecules are 30 and 20.5 Å, respectively; both are neutral. The complete molecule passed neither through the junctions of the insect gland nor through those of the insect cell cultures. The clipped one passed through both. It also passed when the two molecular species were injected together (in mixture) into the cells, showing that the permeation failure of the larger molecule was not due to major permeability alteration.

On the other hand, the junctions of the three mammalian cell cultures were not sensibly permeated by either molecule of the set. However, they were permeated by the 16-Å-wide LRB(Glu)OH (3T3-BALB/c cells) and LRB(Glu)$_2$OH (B and RL cells) when these peptide molecules were injected singly, as shown before (*3*), or together with the glycopeptides.

We conclude that the limiting diameter of the cell-to-cell channel lies between 16 and 20 Å in these mammalian cells and between 20 and 30 Å in the insect cells.

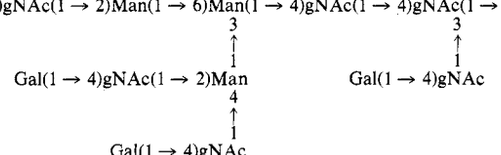
There is evidence that the permeability of the cell-to-cell channel may be modulated in a graded manner by cytoplasmic Ca^{2+} (*14*). Thus, the question arises whether the steric difference reflects a basic (genetic) structural channel difference or merely a modulation in structure due to a difference in the cellular states. This question cannot be strictly answered until all channel-modulating factors are known and the cellular states can be determined in that respect. We are inclined to think that the difference is basic. The insect channels show the same permeability properties whether they are determined in organ or in culture, and the major permeability difference is apparent with eight mammalian cell types in culture, in conditions (probing, cell size) entirely comparable to those of the insect cell cultures (*3*). There are also suggestive differences at the electron microscopic level: the membrane particles of gap junction, the elements widely thought to contain the

Table 1. Cell-to-cell passage of probes.

Probe	Molecular weight	Cell-to-cell passage*				
		Insect		Mammalian culture		
		Chironomus salivary gland	Culture AC-20	B	RL	3T3-BALB/c
		Linear oligosaccharides				
Alpha-linked monomers						
Glucosyl-FSTU†	732	+				
Maltotriosyl-FSTU	1056	+				
Maltotetraosyl-FSTU	1218	+				
Maltopentaosyl-FSTU	1380	+				
Maltohexaosyl-FSTU	1542	+				
Alpha-linked dimers						
Bis(maltohexaosyl)-FBSTU‡	2678	±				
Bis(maltoheptaosyl)-FBSTU	3002	±				
Beta-linked dimers						
Bis(cellobiosyl)-FBSTU	1382	+				
Bis(cellotriosyl)-FBSTU	1706	+				
Sialo-oligosaccharide§-FSTU	1388	+				
		Branched glycopeptides				
FTU-agalactoglycopeptide‖	2327	+	+	−	−	−
LRB-agalactoglycopeptide¶	2449	+				
FTU-glycopeptide#	2975	−	−	−	−	
RB-glycopeptide**	3014	−	−	−	−	−
LRB-glycopeptide**	3097	−				

*A + sign denotes cell-to-cell passage in all trials—in the case of the gland, to all first- and second-order (and often higher order) neighbors of the injected cell, and in the case of the cultures, to ≥ 80 percent of the first-order neighbors. A ± sign denotes relatively slow passage, frequently limited to first-order neighbors, and a − sign denotes no passage. Each positive datum, in gland cells, represents positive passages through ≥ 12 junctions (that is, through all first- and second-order giant cell junctions in each of at least three injection trials); in insect culture cells, passages through about 100 junctions (20 trials). The negative data represent six and seven trials in the gland cells with the FTU- and RB-labeled glycopeptides, respectively; 20 trials with each glycopeptide in the insect culture cells; and 9 to 14 trials with each glycopeptide in the mammalian cells. (The injected cells had two first-order giant cell neighbors in the gland and, on the average, five and four first-order neighbors, respectively, in the insect and mammalian cultures.) Continued permeance of a smaller molecule coinjected with the impermeant served as a control in the case of negative passage. The combinations used for coinjections were FTU-glycopeptide/LRB SO_3 (gland), LRB-glycopeptide/FTU-agalactopeptide (gland and insect culture), FTU-agalactopeptide/LRB(Glu)$_2$ (mammalian B culture), and FTU-agalactopeptide/LRB(Glu) (3T3-BALB/c). †FSTU, *N*-fluoresceinyl-*N'*-1-deoxysorbitylthiourea (570 daltons). ‡FBSTU, *N*-fluoresceinyl-*N'*-bis(1-deoxysorbityl)thiourea (734 daltons). §Gal(β1 → 3)GalNAc(β1 → 4)[NeuAc(α2 → 3)]Gal(β1 → 4)-FSTU. This molecule (with one negative charge) is the only charged sugar on this list. ‖Same as FTU-glycopeptide but without the four terminal galactoses. ¶Same as FTU-agalactoglycopeptide except that label at threonine is LRB.

#Gal(1 → 4)gNAc(1 → 2)Man(1 → 6)Man(1 → 4)gNAc(1 → 4)gNAc(1 → 4)Asn-Thr-NH-FTU.



The structure admits some other positions for the rightmost Gal(1 → 4)GlcNAc residue, introducing an uncertainty of 2 to 3 Å in the dimensions of Fig. 1; the dimensions given are the power limits. **Same as FTU-glycopeptide except that label at threonine is RB or LRB; LRB (the largest label) is less than the width of the glycopeptide; see Fig. 1.

channels, are smaller in mammalian cells than in insect cells or in arthropod cells in general (*15*). The present results also correlate nicely with another aspect of gap-junction particle structure. The central opening occupied by a hydrophilic stain at the intercellular end of the mammalian particles has a diameter of about 20 Å, narrowing toward the particle interior, where it was not resolved (*16*). Although this does not tell about the permeation-limiting channel diameter, it is pleasing that the value is close to the one we obtain by molecular probing.

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9. This was checked by bathing the gland cells, on the basal and luminal sides (the gland lumen was slit open for this purpose), with strongly fluorescent tracer medium for 0.5 to 1 hour and washing them; no fluorescence was detectable in the cells. (Junctional transit times were up to 2 minutes for the largest permeants.)
10. The junction-permeant tracers were incubated for 30 to 60 minutes (that is, 15 to 120 times their junctional transit times) with mashed gland cells in K medium; one would expect maximum lysosome activity in these conditions. The paper electrophoretic profiles of the tracers were the same as before incubation.
11. Lines B and RL are representatives of mammalian cell group I and 3T3-BALB/c of mammalian cell group II. Group I has somewhat less restrictive cell-to-cell channels (*3*). See (*3*) also for media and culture of the mammalian and insect cells.
12. These linear peptides' junctional permeance, nonjunctional membrane impermeance, and stability in cytoplasm have already been shown (*3*).
13. Microinjection into the salivary gland cells was hydraulic (*2*). Injection into the cell cultures was iontophoretic (*3*); the tracers, in KCl solutions, were ejected by solvent drag.
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Plasmid DNA in *Treponema pallidum* (Nichols): Potential for Antibiotic Resistance by Syphilis Bacteria

Abstract. *A plasmid DNA structure (approximate molecular weight = 7.5 × 10^6) was identified in the human pathogen* Treponema pallidum *(Nichols). The inability to isolate this plasmid from rabbit host tissue and the total lack of DNA homology of the plasmid with rabbit DNA has confirmed its* Treponema pallidum *origin. The observation documents a newly recognized and potentially significant genetic capability for* Treponema pallidum.

There is a paucity of information concerning the genetics of the bacterium *Treponema pallidum* (Tp), the etiological agent of syphilis (*1–3*). This is due to the inability to cultivate this organism in vitro (*4*) and to difficulties in the isolation and purification of Tp cells grown in rabbit testicles (*5*). We now report a plasmid DNA component in Tp (Nichols) that was identified during isolation and purification of Tp DNA for cloning studies. To the best of our knowledge, this is the first report demonstrating the presence of plasmid DNA among the *Treponema*. The observation raises concern about the possible future course and pathogenesis of syphilis, especially with regard to the potential acquisition or emergence of extrachromosomally mediated resistance to antibiotics to which wild-type strains have been so exquisitely sensitive.

The Nichols strain of Tp, which was isolated from the cerebrospinal fluid of a syphilitic patient in 1912 (*6*) but still remains pathogenic for humans (*7*), was used as the representative human pathogen for our study. Crude bulk DNA was isolated from this organism and chromatographed over Sephacryl S-300 (Fig. 1) for the routine removal of contaminating RNA fragments (*8*). The elution profile of this DNA on Sephacryl revealed at $A_{254\ nm}$ a peculiar shoulder just prior to the major RNA peak. This shoulder has not been previously observed during the chromatography of other types of prokaryotic and eukaryotic DNA samples prepared in our laboratory. Agarose gel (0.8 percent) electrophoresis of this purified material resolved a faint but sharp DNA band that migrated slightly more slowly than a 23,000-base-pair (1.5 × 10^7 dalton) marker of linear DNA (Fig. 2A, lane 3). The DNA material was isolated several times from independent preparations of purified treponemes, suggesting that a plasmid DNA component had been identified in Tp cells.

The migration of the plasmid DNA band in the agarose gel was consistent with that of many mitochondrial DNA's of eukaryotes (*9*). However, the treponemal origin of the plasmid DNA was supported by our inability to isolate a similar DNA species from testicles of sham-infected rabbits. We were also unsuccessful in purifying an analogous DNA component from approximately 3 × 10^8 rabbit testicular fibroblast tissue culture

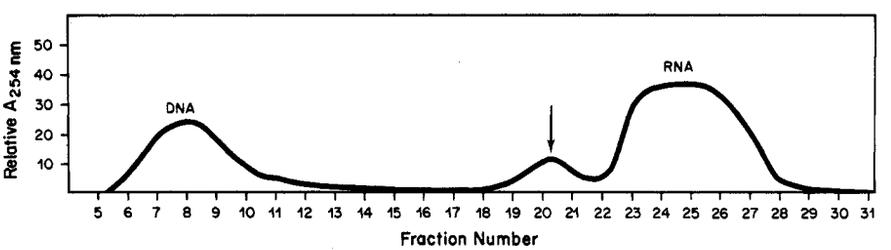

Fig. 1. Sephacryl S-300 column chromatography of crude *T. pallidum* bulk DNA. The *T. pallidum* was cultivated in the testicles of five cortisone-treated rabbits (*20*). Treponemal cells were extracted from diced testicles aerobically in 50 ml of phosphate-buffered saline (*p*H 7.2) at 23°C for 30 minutes (rotary shaking at 180 rev/min). Rabbit tissue debris was removed from the treponemal suspensions by centrifugating twice at 500*g* for 10 minutes. Treponemes were then collected by centrifugation at 16,000*g* for 30 minutes and washed twice by centrifugation in hypotonic lysis buffer (HLB) (25 m*M* tris-HCl, 10 m*M* EDTA, *p*H 8.0) to liberate and remove the DNA of the remaining intact testicular cells (*21*). Enumeration by dark-field microscopy (*22*) indicated a recovery of approximately 2 × 10^{10} intact treponemes, which were first treated with lysozyme (100 μg/ml) for 10 minutes at room temperature (*23*) in 8.5 ml of HLB and then lysed and incubated (37°C, 5 minutes) in the presence of ribonuclease A (50 μg/ml) and 0.8 percent sodium tri-isopropylnaphthalenesulfonate (*15*). The lysate was made 0.2*M* with respect to NaCl and extracted with phenol; the crude treponemal DNA was precipitated at − 20°C by the addition of two volumes of ethanol and allowed to stand overnight before collection by centrifugation at 23,500*g* for 45 minutes. The bulk DNA was dried, dissolved in 1 ml of buffer (10 m*M* tris-HCl, 10 m*M* NaCl, 1 m*M* EDTA, *p*H 7.6), and chromatographed on Sephacryl S-300 to remove RNA fragments (*8*). The arrow indicates an elution profile shoulder (*T. pallidum* plasmid DNA) not normally observed for other DNA's (*8*).

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