tions of each membrane fraction were incubated under standard conditions for studying membrane protein phosphorylation, and the incorporation of ^{32}P-labeled phosphate into membrane protein was measured as described previously (*4–6*). Benzodiazepines were routinely dissolved in 100 percent dimethyl sulfoxide (DMSO) and added to the membrane suspension at a dilution of 1:500. Control mixtures received an equal amount of DMSO.

15. B10(+) and B10(−) represent the pharmacologically active (+) and inactive (−) benzodiazepine enantiomers corresponding to Ro11-6896 and Ro11-6893, respectively.
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17. Potencies of benzodiazepine kinase inhibition (represented by K_i values) were compared to the relative pharmacological potencies of these drugs in several test systems: inhibition of maximum electric shock–induced seizures in mice, cat muscle relaxant action, inhibition of electric shock–induced fighting of mice, antagonism of pentylenetetrazol-induced convulsions in mice, impairment of mouse rotorod performance, inhibition of rat and monkey conditioned avoidance [G. Zbinden and L. O. Randall, *Adv. Pharmacol.* **5**, 213 (1967); L. O. Randall, W. Schallek, L. H. Sternbach, R. Y. Ning, in *Psychopharmacological Agents*, M. Gordon, Ed. (Academic Press, New York, 1974), vol. 3, p. 175]. Correlation coefficients and statistical significance for each test system in comparison to kinase inhibition was determined for Ro5-5345, Ro5-5807, Ro5-2180, diazepam, clonazepam, oxazepam, nitrazepam, chlordiazepoxide, Ro5-4864, and flurazepam by standard procedures used to compare the affinity of the benzodiazepine receptor to pharmacological potency [H. Mohler and T. Okada (*3*); C. Braestrup and R. Squires, *Eur. J. Pharmacol.* **48**, 263 (1978)]. These references were also used to obtain statistically significant correlation of potency of membrane receptor affinity and pharmacologic potency. The median effective dose for Ro5-4864 in inhibiting maximum electric shock–induced seizures in mice is 36.1 mg/kg. These data were obtained from Dr. W. E. Scott and represents experimental work done at Hoffmann–La Roche, Inc.
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* Reprint requests and correspondence to R.J.D.

4 March 1981; revised 20 May 1981

Nullisomic *Tetrahymena:* Eliminating Germinal Chromosomes

Abstract. *Germinal and somatic functions in* Tetrahymena *are performed separately by the micro- and macronuclei, respectively. Cells with haploid micronuclei were mated with diploids to yield monosomic progeny. These were induced to undergo a form of self-fertilization, generating cells lacking both copies of one or more of the five chromosomes in the micronucleus while still possessing a complete macronuclear genome.*

Fertile strains of the ciliate *Tetrahymena thermophila* have two nuclei. The germinal micronucleus determines inheritance in the next sexual generation and the somatic macronucleus directs the phenotype of the cell. We recently recognized that the two nuclei can be manipulated independently; we can create heterokaryons with different micro- and macronuclear genotypes (*1*). The phenotype of these heterokaryons always reflects the genotype of the macronucleus. The micronucleus remains silent.

We have now exploited this micronuclear silence to produce strains with micronuclei missing both copies of one of the five chromosomes. These nullisomics are viable because they have a normal macronucleus. They are extremely useful because, when crossed with a diploid, they yield progeny with monosomic micro- and macronuclei, facilitating genetic analyses of that chromosome. Since *T. thermophila* has a haploid set of five chromosomes (*2*), it should require the establishment of only five unique strains to have nullisomics for each of the chromosomes. Figure 1, A and B, demonstrates that the chromosomes in a normal diploid can readily be visualized during meiosis; identification of abnormal karyotypes is feasible.

Variations in micronuclear chromosome content can occur if meiotic missegregation is induced. One method for generating such meiotic misbehavior became possible when Nanney and Preparata (*3*) isolated cells with normal macronuclei but haploid micronuclei. Of importance here is that these cells still attempt to mate. During conjugation the haploid micronucleus undergoes meiosis, but lack of pairing partners for each of the chromosomes results in the generation of meiotic products that contain variable numbers of chromosomes. Thus crossing a haploid with a diploid should yield monosomics.

To isolate successful progeny of such a cross, a haploid strain was constructed which contained in its micronucleus the dominant cycloheximide (cy) resistance mutation *Chx* (*4*). It was crossed to a strain that had a micronucleus homozygous for the *Mpr* allele, a dominant mutation which, when expressed, confers resistance to 6-methylpurine (6-mp) (*5*). Progeny were selected by growing exconjugants in both cy and 6-mp; single cells were cloned following the drug selection.

Many of these progeny clones should be aneuploid. Cytological examination of the micronuclei during meiosis was employed to detect the monosomic clones. However, as can be seen in Fig. 1A, only the haploid number of chromosomes is evident during meiotic prophase in a diploid cell, since homologous chromosomes pair so completely. Monosomics can only be clearly identified at anaphase I by resolving all the chromosomes at both poles. To simplify the cytogenetic identification of monosomics, the progeny of the haploid-diploid cross were crossed to strain A*, and exconjugants were cloned to create nullisomics. Crosses to A* undergo an abnormal form of conjugation called genomic exclusion (*6*). In these crosses the non-A* mate undergoes the normal events preceding fertilization: meiosis, elimination of three of the four products, mitotic doubling of the retained haploid nucleus, and transfer of one of these to its partner. In contrast, A* has a defective micronucleus that is lost at meiosis. After transfer, the only germinal genome in each of the two cells is contained in the haploid nucleus from the non-A* parent. This zygote nucleus undergoes an endoreduplication (*7*); each conjugant now has an identical, fully homozygous genome. Thus the loss of the A* micronucleus leads to uniparental micronuclear inheritance; the new genome is homozygous and comes only from the non-A* parent. The next step in normal conjugation, development of a macronucleus from the zygote nucleus, fails to occur. The end result of this round of mating (round 1) is the generation, from each pair, of two cells with identical homozygous micronuclei derived solely from the non-A* parent, but with parental macronuclei (*8*). Thus a change in the germinal genome has been effected, but the cells' phenotypes (drug sensitivity, mating type, and so on) are unaltered.

When monosomics are crossed to A* and pairs of exconjugant clones established, the micronuclei of each pair of round 1 exconjugants should be fully homozygous, identical, and nullisomic or diploid for each chromosome pair. The nullisomics should still be viable, because the macronuclei are still parental. When the round 1 exconjugants are allowed to remate (round 2), they now not only undergo normal biparental fertilization, they also develop new macronuclei (*6*). Thus, if the original isolate from the double drug selection were monosomic, one would expect half the isolated pairs of round 1 exconjugant clones to be diploid and yield viable round 2 progeny and the other half to be

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nullisomic and always die after round 2. Results of round 2 mating can therefore be used to identify the round 1 exconjugants that have diploid micronuclei and those that have nullisomic micronuclei.

Among the clones of selected cells resulting from the haploid-diploid cross, one yielded exconjugants capable of producing round 2 progeny—but only in about 10 percent of the pairs of exconjugant clones. This suggested that the isolate might be a multiple monosomic. This was confirmed by separately cloning several round 1 exconjugants from this line and fixing them for cytological examination during meiosis. Figure 1C shows a clone that gave viable progeny at round 2; it is clearly diploid. Figure 1, D and E, shows other round 1 clones from this same monosomic line which could not yield viable progeny at round 2; one is a single nullisomic, the other is a double nullisomic. Using this test, we observed that all round 1 clones from this particular line were either diploid, single nullisomic, or double nullisomic. This is exactly what is expected of round 1 clones of a double monosomic.

One of the resulting single nullisomic round 1 strains was missing the smallest chromosome, which we have termed number 5. Although it could not yield viable round 2 progeny, it did give viable progeny when crossed with wild type. Since these progeny were resistant to both cy and 6-mp, the nullisomic strain must have been homozygous for both these markers. Bearing in mind that the nullisomic retains the previous generation's macronucleus, we propose to indicate its genotype and phenotype as *Chx/Chx Mpr/Mpr* Nulli 5 (cy r, 6-mp r). In keeping with convention (*1*), the three-letter symbols not included in parentheses indicate the micronuclear genotype, with a capital first letter indicating a dominant mutation; the symbols inside the parentheses describe the macronuclear phenotype. The phenotype is purposely written with symbols other than those used for the micronuclear genotype, since the exact macronuclear genotype is not known; all that can be assayed is the phenotype.

The word Nulli will be used in the micronuclear genotypic description, followed by an arabic numeral to designate the chromosome or chromosomes involved. We propose that the five chromosomes be designated by arabic numerals, with 1 and 2 for the largest (two large metacentrics, so far indistinguishable), 3 and 4 for the next largest (again two metacentric chromosomes that are very nearly the same size), and 5 for the smallest (a telocentric) (*9*).

This strain was further manipulated to yield strains with a macronucleus expressing sensitivity to both drugs, a micronucleus homozygous for the *Chx* or the *Mpr* mutation (but not both), and nullisomic for chromosome 5. The *Chx/Chx Mpr/Mpr* Nulli 5 (cy r, 6-mp r) strain was crossed with wild type and successful exconjugants were isolated. Progeny were immature (*10*) and resistant to both drugs. Soon after mating, the polyploid macronucleus is a mosaic of the parental alleles. Since this nucleus divides amitotically, it apparently has no mechanism to ensure that both parental alleles segregate in equal numbers at division; macronuclei result which express the phenotype of only one of the members of each allelic pair. This phenomenon, called phenotypic assortment (*11*), allowed us to isolate subclones that stably expressed the recessive wild-type phenotypes of sensitivity to both drugs. Since these isolates still had micronuclei that were heterozygous for the two drug markers and monosomic for chromosome 5, they were crossed to strain A* and round 1 pairs were isolated. These clones had one of eight possible micronuclear genotypes (homozygous for one of the two alleles at each of the two drug loci and diploid or nullisomic for chromosome 5), but they retained the assorted wild-type macronucleus of the previous generation. Clones containing the two desired genotypes were identified by (i) crossing with wild type and assaying for expression of the appropriate drug resistance and (ii) crossing the round 1 progeny with each other (round 2) and identifying those lines that could not yield drug resistance (failed to make progeny).

Two clones with the two desired genotypes were retained. These were again examined cytologically at meiosis, and, as shown in Fig. 1, F and G, both copies of the smallest chromosome were again

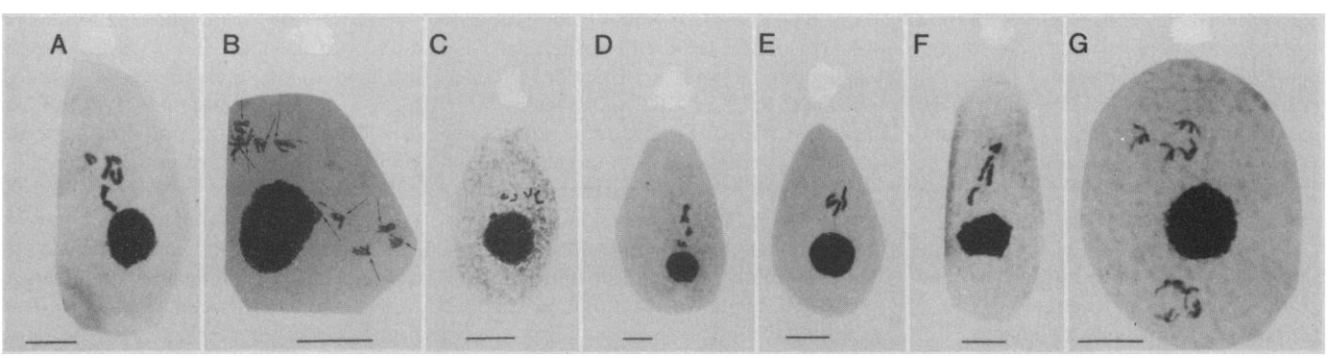

Fig. 1. Chromosome characteristics of normal and abnormal *Tetrahymena* prepared during meiosis. The unknown strain was mated to strain A* at 30°C (*15*). Since A* loses its micronucleus during meiosis, the identity of each conjugant in every pair could be determined; only the non-A* partner is shown here. Five hours after mixing the starved parents, 1 ml of cells was centrifuged at 160*g* for 1 to 2 minutes and most of the supernatant was removed. The remaining thick suspension of cells was squirted into 1 ml of fixative [99 parts Schaudinn's (*16*) and 1 part glacial acetic acid] and left for 1 hour at room temperature. Then the suspension was centrifuged, the supernatant discarded, and the pellet resuspended in 1 ml of 70 percent ethanol; it may be kept in the alcohol for several minutes to several days with no apparent difference in subsequent staining. For staining, 0.5 ml of the fixed sample was centrifuged, the ethanol discarded, and the pellet resuspended in 0.5 ml of a 3:1 mixture of methanol and acetic acid. The cells were immediately centrifuged and the supernatant was aspirated to leave a thick suspension of cells (5 to 6 drops). The cells were then dropped onto a microscope slide from a distance of at least 1 foot and air-dried. The preparations were hydrolyzed in fresh 5*N* HCl for 1 to 2 minutes at room temperature, rinsed in distilled water, and air-dried. They were then immersed in 10 m*M* sodium phosphate buffer (*p*H 6.8), and sufficient Giemsa stain was added to make a 4 percent solution. After 10 to 15 minutes at room temperature the stain was removed by flushing with distilled water, the slides were air-dried, and cover slips were mounted. (A) Prophase and (B) late anaphase in a diploid. The arrows in (B) point to what appears to be the centromeric regions for the five chromosomes. (C, D, and E) Prophase in a reconstituted diploid, single nullisomic, and double nullisomic, respectively. All were obtained from round 1 genomic exclusion of a double monosomic strain. (F) Prophase and (G) late anaphase I in a single nullisomic designed to have a wild-type macronucleus but a micronucleus with a dominant selectable marker. The bars all equal 10 μm. The large heavily stained object is the macronucleus.

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*).

PETER J. BRUNS
TRUDY E. B. BRUSSARD
Section of Genetics and Development, Cornell University, Ithaca, New York 14853

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28 April 1981

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.

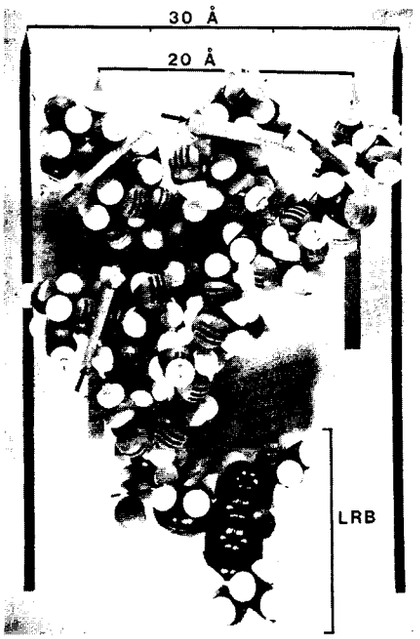

Fig. 1. Space-filling model of the branched glycopeptide tracer molecules. Shown is the LRB-labeled glycopeptide. The four terminal galactoses of this molecule are missing in the agalactopeptide; the planes (arrows) indicate where the galactoses are clipped off in the preparation of this molecule. The 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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