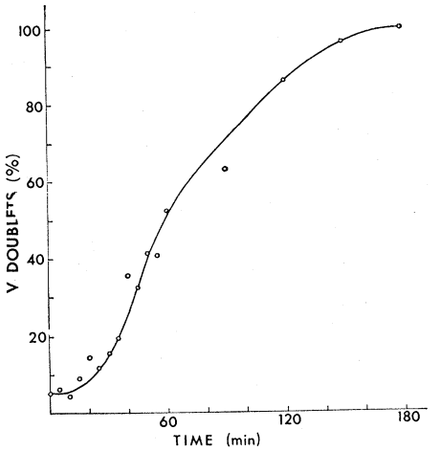

Fig. 3. Percentage of V-shaped doublets in cells of a culture of *S. pombe* exposed to Colcemid as in Fig. 1.

yeast microcolonies by their more rapid growth. The drug concentration used is not fully inhibitory, so that the spontaneous mutation rate per cell division need not be unusually high for this frequency of appearance. Other isolates were made by first enriching for growth of resistant cells in liquid YEG medium containing $5 \times 10^{-3}M$ Colcemid. When the presumptive mutant colonies are picked and streaked on YEG-Colcemid plates, colonies are readily visible in 1 to 2 days and are 2 mm in diameter by 3 days. A similar tolerance to Colcemid in YEG agar is shown by mutants previously grown on YEG agar alone, and hence the resistance is an inherited trait. The residual sensitivity to Colcemid of different mutants is readily measured by streaking them along YEG plates containing a lateral gradient of drug concentrations (*13*). Concentrations tolerated by the mutants are estimated by the range of the gradient which permits yeast growth. Resistance among mutants varies (up to $10^{-2}M$ Colcemid) by this definition.

Unlike colonies of sensitive cells, colonies of mutants on YEG agar contain single cells, numerous cell doublets, and cohesive aggregates of cells. When the mutants are grown on YEG agar with Colcemid, elongated cells with several division plates are frequent. Fortunately, the mutants have retained normal characteristics of fertility and ascospore formation when crossed with wild-type cells, and they appear to be suitable for further genetic analysis.

Our observations with *S. pombe* suggest that it may be suitable for a study of mitosis in which antimitotic agents are used. Its choice on the basis of cell division characteristics proved fortuitous since a priori there was no reason to believe it would be more sensitive than other Ascomycetes. The inertness of other yeast species to Colcemid is unexplained, and in a formal sense they behave as Colcemid-resistant strains.

Cytological examinations of *S. pombe* and of other yeasts have revealed microtubular elements within the nucleus (*14*). A presumptive mitotic target for the colchicine alkaloids appears to be present, although it remains to be isolated and more directly demonstrated. The main argument for a mitotic apparatus in yeast species still rests on the need for a mechanism to carry out the observed equipartite distribution of a number of genetic linkage groups. The extent to which the responsible structure in yeast resembles that of higher cells remains to be determined. The immediate question that we are led to ask is whether Colcemid inhibits cell division in *S. pombe* by interacting with a mitotic apparatus, or whether the observed inhibition is secondary to some other property of the drug.

Caution is warranted on the interpretation of the resistant mutants. The explanations that must be considered include permeability changes and detoxification mechanisms as well as structural changes in the mitotic apparatus which might affect either the binding of Colcemid or the functioning of a possible complex between Colcemid and the mitotic apparatus. There is a possible analogy with streptomycin resistance in bacteria, where there are separate mechanisms involving either a detoxifying modification of the drug or a change in a protein subunit of the bacterial 30*S* ribosome, so that protein synthesis is no longer inhibited by the drug (*15*). Until we can assign appropriate mechanisms of resistance, we propose the designation *cid* for mutants which differ from wild-type in *N*-deacetyl-*N*-methylcolchicine *i*nhibition of *d*ivision.

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Induction and Isolation of Auxotrophic Mutants in Somatic Cell Cultures of Nicotiana tabacum

Abstract. *A method for the selection of auxotrophic mutants from somatic cell cultures of* Nicotiana tabacum *depends upon the incorporation of 5-bromodeoxyuridine into the DNA of wild-type cells and upon its lack of incorporation into the DNA of auxotrophic cells. There may be more than one functional copy of essential genes in the haploid genome of* Nicotiana tabacum.

The present work was undertaken to develop methods for the isolation of auxotrophic mutants from somatic cell cultures of higher plants. Recent developments in plant tissue culture research indicate that haploid tissue is easily obtained from anther culture of several solanaceous species (*1*) and that single tobacco cells in culture can be treated as experimental organisms (*2*). A previous paper describes a technique for the isolation of auxotrophic mutants in the haploid generation of ferns (*3*). This report describes the successful utilization of the mutant isolation technique on single haploid tobacco cells in culture.

The selective technique employed is

Table 1. Culture mediums. Additions to the mineral salts and organic constituents described by Linsmaier and Skoog (*5*). Mediums 5 and 6 were used to plate single cells. Single-cell suspensions were mixed with the semisolid agar and poured into a petri dish. At a density of approximately 2.5×10^3 cells per milliliter, plating efficiency on medium 5 ranged from 37 to 56 percent, whereas that on medium 6 was 88 percent. Medium 6 also contained $10^{-5}M$ thymidine, 800 mg of casein hydrolyzate per liter, and 400 mg of yeast extract per liter. Cells treated with mutagen and BUdR were plated in this medium at a density of 2.5×10^3 cells per milliliter.

Medium	Agar (%)	Sucrose (%)	Indole-3-acetic acid (mg/liter)	Kinetin (mg/liter)
1	0.8	4.0	0.1	
2	0.8	4.0	2.0	0.3
3		4.0	2.0	0.3
4		4.0	2.0	0.03
5	0.5	4.0	2.0	0.3
6	0.5	2.0	2.0	0.3

modeled after a system designed for use with mammalian cell cultures (*4*). A mixed population of auxotrophic and wild-type cells is placed in an unsupplemented medium where wild-type cells can grow but auxotrophs cannot. The cells are then exposed to a compound which will kill only dividing cells. In a typical experiment a large population of single haploid cells is exposed to a mutagen and then grown on a minimum medium for several cell generations. This treatment serves to deplete the pool of growth-limiting metabolite present in the individual mutant cell and halt its growth. Nonmutant cells continue to grow and divide on the minimum medium. This mixed population of cells is exposed to 5-bromodeoxyuridine (BUdR). Growing cells incorporate BUdR into their DNA and are subsequently killed by exposure to visible light. The nondividing mutant cells do not incorporate BUdR and so are not killed by the light. Auxotrophs are then recovered by culturing them on a nutritionally supplemented medium which permits their growth into callus masses. The individual masses are isolated and their nutritional requirements determined. Plants are subsequently induced to differentiate from the auxotroph calluses.

The basic medium (Table 1) used in these experiments was that of Linsmaier and Skoog (*5*). All work with liquid mediums was done on a rotary shaker operated at 180 cycles per minute. Unless indicated, all cultures were grown under approximately 4000 lux with a 16-hour light–8-hour dark cycle. Culture temperature was 25° ± 1°C.

Haploid plants were produced by placing late tetrad stage anthers of *Nicotiana tabacum* (L.) var. "Wisconsin 38" on medium 1. Haploid plants developed from approximately 12 percent of the anthers. Haploid stem tissue was induced to form callus by placing longitudinally sliced apical sections on medium 2. The rare resulting haploid callus formed rudimentary shoots and leaves on this medium. The stem sections more commonly gave rise to diploid and tetraploid calluses which were typically nondifferentiated, and friable. The haploid callus was routinely cultured on medium 3. Chromosome counts indicate that the haploid tissue has remained stable after 6 months in culture. The tissue has also retained its differentiation into rudimentary shoots and leaves. Experiments with varying hormone concentrations demonstrate that, as expected (*6*), a higher concentration of kinetin favors the growth and differentiation of shoots and leaves, whereas a higher concentration of indole-3-acetic acid favors the formation of nonfriable callus. Free haploid cells were obtained by placing a piece of the differentiated callus into medium 4. After several weeks pieces of nonfriable callus appeared and were subcultured on medium 3; this produced a more friable mass from which haploid cell suspensions were obtained. Single cells and small groups of cells were isolated by using a filter of four layers of cheesecloth. Haploid cells constituted 65 to 80 percent of this suspension as determined by chromosome counts. The average generation time of the cells under these conditions ranged from 38 to 52 hours.

The mutant isolation technique requires that all mutant cells have ceased DNA synthesis and that nonmutant cells continue synthesis and incorporate enough BUdR into their DNA so that they are killed by subsequent exposure to visible light. To determine how long an auxotrophic cell continues to divide after a mutation has occurred, a population of single haploid cells was exposed to a dose of the mutagen ethyl methanesulfonate (EMS) which resulted in 99.99 percent lethality. These cells were immediately plated in medium 5. The average colony size formed by those cells which continued to divide was almost four cells. This constitutes two cell generations. Since the generation time of the haploid cells is from 38 to 52 hours, some mutant cells continue to divide, and presumably synthesize DNA, for up to 4 days. Thus mutagen-treated cells were routinely starved for 96 hours before BUdR treatment. To determine the survival of nonmutant cells treated with BUdR, a population of single haploid cells was grown in the dark on medium 3 supplemented with $10^{-5}M$ BUdR. Portions were removed at various time intervals; the cells were plated in medium 5 and illuminated by placing them 30 cm from a bank of four 40-watt cool white fluorescent lamps for at least 48 hours. After 36 hours of incubation in BUdR, no cells survived.

Large populations of haploid cells were treated with 0.25 percent EMS for 1 hour. Cell survival ranged from 46 to 67 percent. The cells were washed twice in medium 3 and then resuspended in fresh medium 3. The population was incubated for 96 hours with fresh medium added at 48 hours. The medium was supplemented with BUdR to a final concentration of $10^{-5}M$ and kept in the dark for 36 hours. At the end of the dark incubation period the cells were washed twice with medium 3, plated on medium 6, and illuminated with cool white fluorescent lamps. Calluses which

Table 2. Auxotrophic mutants of *Nicotiana tabacum*. Initial weight of tissue for growth tests was approximately 100 mg. Auxotrophic supplements were supplied as follows: all amino acids, 100 μg/ml; all nucleic acid bases, 50 μg/ml; choline chloride, 1.0 μg/ml; *para*-amino benzoic acid, riboflavin, pyridoxine HCl, nicotinic acid, and folic acid, 0.2 μg/ml, respectively. Vitamins were sterilized by filtration and added to previously autoclaved medium.

Isolation number	Required supplement	Increase in wet weight in 3 weeks (%)	
		Minimum medium	Minimum medium plus required supplement
11	Hypoxanthine	49	274
82	Biotin	131	257
25	*para*-Amino benzoic acid	100	237
67	Arginine	73	193
116	Lysine	44	204
60	Proline	36	212
Nonmutant haploid callus No. 1		234	
Nonmutant haploid callus No. 2		271	
Nonmutant haploid callus No. 3		288	

appeared on this medium were isolated. Those calluses which were not able to maintain a normal rate of growth on unsupplemented medium were tested on a number of different nutritional supplements in medium 2, as outlined by Holliday (*7*), to determine their auxotrophic requirement.

Over 1.75×10^6 haploid cells were examined. Of the 119 calluses that were isolated only six proved to be auxotrophic (Table 2). There is little or no restriction on the auxotrophic types which can be recovered since auxotrophs for nucleic acid bases, vitamins, and amino acids have been observed. All six auxotrophic calluses retained their haploid chromosomal constitution. Plants were differentiated from four of the mutant calluses.

It is significant that so few mutants were isolated and that these mutants all proved to be leaky; for they continued to grow slowly on unsupplemented medium. This is in contradistinction to similar work with ferns (*3*). One explanation may be that the selection procedure is not able to detect nonleaky auxotrophs. This could occur because either the low plating efficiency or one of the experimental parameters is not permitting auxotrophs to survive selection. Mutant single cells might be killed by the long starvation period designed only to inhibit their growth. However, this explanation is partially excluded by further experiments with only a 6-hour starvation period. In these experiments no increase in the number of auxotrophic or nonauxotrophic cells surviving the treatment with BUdR and near-visible light was observed. Another explanation may be that nonleaky mutants are not being induced.

Because *N. tabacum* is an allopolyploid, the haploid cell may actually contain two copies of essential genes. The low number and leaky quality of the auxotrophic mutants could be due to a lack of functional diploidization of the *N. tabacum* genome, and thus a lack of functional haploidization in the somatic haploid cell. Although Smith (*8*) noted that most morphological mutants of *N. tabacum* act as if the species is a functional diploid, this explanation may not be valid for loci involved with the metabolism of essential nutrients. Clausen and Cameron (*9*) noted that the morphological characters hairy filaments and yellow burley were each determined by duplicate factors belonging to different parental genomes of the allopolyploid *N. tabacum*. This evidence argues that the functional diploidization of the *N. tabacum* genome is not complete. The auxotrophic mutant characteristics appear best explained by the assumption of incomplete diploidization. Thus, there may be more than one functional copy of metabolically important genes in the haploid genome of *N. tabacum*.

The data on the growth of the auxotrophic mutants further implies a physiological differentiation between the two functional copies of a duplicated gene. If the two copies are identical, then a mutation in one of them would not be expected to appear as a mutant, inasmuch as a low, residual amount of enzymatic activity will still permit wild-type amounts of growth in other eukaryotes. A mutation in one of a pair of duplicate genes should not reduce the normal growth rate by more than half. Such a reduction is found only with auxotroph number 82. The remaining five auxotrophs show much slower rates of growth. This phenomenon could be explained by assuming either that the two gene copies are subject to different regulatory controls or that the copies are differentiated so that they operate in different pools or compartments within a cell.

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Trichromatic Mechanisms in Single Cortical Neurons

Abstract. *By chromatic adaptation, all three cone mechanisms of rhesus monkey vision can be identified in single neurons of striate cortex. This trichromatic interaction occurs in cells sensitive to color and indicates that striate cortical cells tend to be more wavelength discriminating than cells at lower stages of the primate visual system.*

Color vision in man (*1*) and in certain primates (*2*) is trichromatic because only three variables are required to produce all color sensations. This trichromacy depends upon the photopigment molecules in the outer segments of cone receptor cells. There are three types of cones, each of which absorbs wavelengths of light differently; one is more sensitive in the blue, another in the green, and a third near the red region of the visible spectrum (*3*). The outputs of these three channels are analyzed by the remainder of the visual system to produce a variety of color impressions.

Knowledge of the processing of color information in the primate central nervous system is confined mostly to the retina (*4, 5*) and the lateral geniculate nucleus (*6, 7*). Less is known about color vision in other areas of the visual nervous system such as the superior colliculus (*8*) and the cerebral cortex (*9, 10*).

In the retina and lateral geniculate nucleus of many vertebrates there are two major types of neurons, the on-center and the off-center cells. Although both types receive excitatory and inhibitory signals, the spatial distributions of these excitatory and inhibitory mechanisms differ over their receptive fields (*11*). An on-center cell receives relatively more excitation and an off-center cell relatively more inhibition in the center of its receptive field. On-center cells are excited and off-center cells are inhibited when light is turned on in the center of their receptive fields; the converse can occur when the light is turned off.

Animals with color vision have cells in which the spectral characteristics of these excitatory and inhibitory processes also differ; that is, a cell is excited by one wavelength and inhibited by another (*4–7, 12*). Such cells are called spectrally opponent, and they are usually on- or off-center cells. Spectrally opponent cells seem to represent an essential step in color discrimination by the vertebrate central nervous system.

In the rhesus monkey, whose color vision is similar to man's, such spectrally opponent cells are abundant in the