utes later the eggs were washed two times with excess leucine-C^{12} and resuspended in sea water containing excess leucine-C^{12} until the M.A. was isolated. If the labeled material observed in the previous experiments were due to adsorbed leucine-C^{14} or to independent nascent proteins formed upon ribosomes within it, the M.A. isolated in this experiment should not be labeled (*8*). Since the M.A. isolated after this treatment was still radioactive, we believe that the labeled protein was either part of the M.A. or that the proteins are retained on the ribosomes for a longer period in sea urchin eggs than in other systems. The latter possibility we consider unlikely because leucine-C^{14} was rapidly incorporated into proteins after its addition to the system.

If the transfer-ribonucleic acid binds the growing peptide chain to the ribosome by covalent bonds (*9*), then hydrolyzing the RNA by treatment with trichloroacetic acid should release nascent protein from the ribosome and allow the protein to be washed away. In the second experiment, the mitotic apparatus isolated from cells grown continuously in leucine-C^{14} was washed as described, and treated with trichloroacetic acid at 90°C for 15 minutes to remove nucleic acids. The M.A. fraction was then washed in the presence of excess leucine-C^{12}, two times in $5 \times 10^{-4}M$ $CaCl_2$, three times in distilled water, applied to albuminized slides,and radioautographed. The M.A. retained its achromatic structure, and the label (Fig. 3) remained associated with the M.A.

The mitotic apparatus may be considered as both a cell organelle and a region of the cell (*1*, p. 236). However, the M.A. probably consists primarily of material directly concerned with cell division, for during its formation particles such as mitochondria and yolk are pushed outside its boundaries (*10*) and proteins isolated from it are relatively few and are present as discrete, homogeneous components (*11*).

Although we cannot state that in our experiments leucine-C^{14} was incorporated into the structural protein of the M.A., we can say that leucine-C^{14} was incorporated into protein present in the M.A. isolated by our techniques, and that the labeled material was retained after treatments designed to remove free amino acids and protein bound to ribosomes. Our results are consistent with the idea that part of the M.A. proteins in sea urchin eggs is synthesized between fertilization and metaphase of the first division.

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Allelic Mapping in Yeast by X-ray–Induced Mitotic Reversion

Abstract. *A new method for determining the sequence of mutational sites is based on the linear dose-effect relation for x-ray induction of allelic recombination in* Saccharomyces cerevisiae. *Mutations at two loci have been mapped by this method. The use of x-ray simplifies allelic mapping and greatly increases its sensitivity.*

When two independently isolated mutant alleles of a given locus, a_1 and a_2, are placed in repulsion in the same diploid yeast cell (a_1/a_2), this heteroallelic diploid has a much higher frequency of reversion to wild type during mitotic division than either of the homoallelic combinations (a_1/a_1 or a_2/a_2). A process of allelic recombination in heteroallelic diploids has been suggested to account for this greater frequency (*1*). The effect can be stimulated both by ultraviolet light (*2*) and by x-rays (*3*). While in the case of ultraviolet light a nonlinear dose-effect relation is observed, with sublethal doses of x-rays the number of induced revertants is proportional to the dose. We have found that the value of the slope of the x-ray curve depends on the pair of alleles involved. The nature of this dependence provides the basis of a new method for determining the sequence of alleles within a gene. We have tested this method at two nutritional loci in *Saccharomyces cerevisiae:* ar_4 (arginine biosynthesis) and tr_5 (tryptophan synthetase).

The strains used in these experiments were cultured in 10 ml of rich liquid medium (2 percent yeast extract, 4 percent peptone, and 4 percent dextrose) to avoid preferential selection of revertants (prototrophs) which occur spontaneously during growth. Cultures were vigorously swirled in 50-ml erlenmeyer flasks for 3 days at 30°C. Between 10^6 and 10^8 washed cells, the number depending on the alleles and the dose, were plated (four plates per dose point) on synthetic medium lacking either arginine or tryptophan. For each dose about 200 cells were plated on each of four plates of complete medium to assay viability. X-rays from a beryllium-window tube (Machlett OEG 60) were delivered to the cells on the agar surface. The x-ray tube was operated at 50 kv (peak) and 20 ma without additional filtration, giving a dose rate of about 200 r/sec at the position of the cells. Visible colonies were counted after 3 to 5 days of incubation at 30°C. Less than 1 percent additional colonies appeared after 5 days of incubation.

Dose-frequency curves for diploids with various allelic combinations at the ar_4 locus are shown in Fig. 1*A*. The slopes of these curves are highly reproducible from one culture of the same strain to another, while the intercept is extremely variable because of the clonal distribution of spontaneous revertants (*4*).

The allelic map in Fig. 1*B* was constructed from the data in Fig. 1*A* on the basis of the assumption that the slope of each curve is proportional to the distance between the two alleles involved. The map intervals so determined are consistently additive.

Figure 2 is a map, constructed by the same method, of several alleles at the tr_5 locus. Again, the intervals are consistently additive, permitting the sequence to be unambiguously determined.

The sequence assigned to the ar_4 alleles has also been confirmed by an entirely independent method. A *cis-*

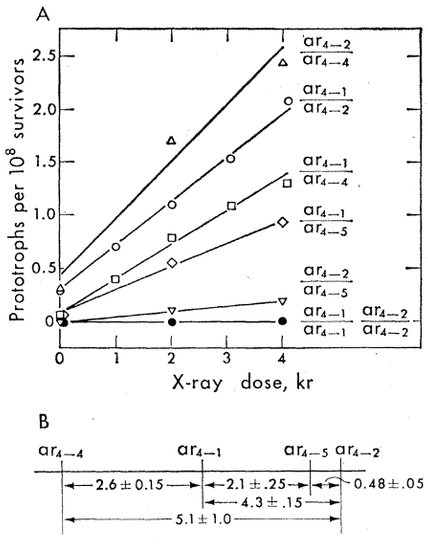

Fig. 1. *A*, Frequency of arginine-independent revertants, as a function of x-ray dose, for various heteroallelic and homoallelic diploid combinations at the ar_4 locus. *B*, Partial allelic map of ar_4 locus in *Saccharomyces* constructed from the slopes of the corresponding curves in *A*. Map intervals are in units of protrotrophs per 10^8 survivors per roentgen. Standard deviations were calculated by the method of least squares.

double mutant $ar_{4-1}ar_{4-2}$ spore culture has been isolated by micromanipulation from asci of an ar_{4-1}/ar_{4-2} heteroallelic diploid, the double mutant strand evidently arising from reciprocal crossing-over between the two alleles. The diploid $ar_{4-1}ar_{4-2}/ar_{4-4}$ reverts to wild type spontaneously (at mitosis) with a high frequency, characteristic of heteroallelic strains, but the $ar_{4-1}ar_{4-2}/ar_{4-5}$ combination reverts at a frequency that is more than an order of magnitude smaller. This is exactly the behavior predicted by the sequence deduced from x-ray data (Fig. 1*B*), since a double exchange would be required to produce a prototroph in the latter case, whereas a single event would suffice in the former.

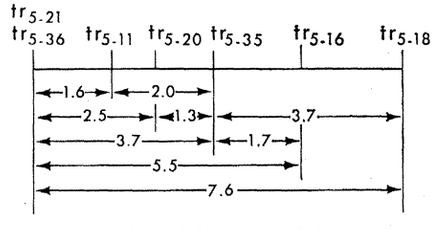

Fig. 2. Partial allelic map of the tr_5 locus in *Saccharomyces* constructed by the x-ray mapping method illustrated in Fig. 1.

The results of additional experiments at the tr_5 locus allow us to relate the map units of the x-ray method (1 unit = 1 prototroph per 10^8 survivors per roentgen) to molecular dimensions. We have mapped more than 30 mutants at this locus. It is likely that the two alleles that are farthest apart are located near the ends of the gene, and we may consider the distance between them to be a minimum estimate of the length of the gene in x-ray mapping units. This distance, 10 prototrophs per 10^8 survivors per roentgen, can then be compared with the size of the tryptophan synthetase molecule. Carsiotis *et al.* (*5*) found the molecular weight of *Neurospora* tryptophan synthetase to be about 122,000. Their studies of properties of the enzyme suggest a quaternary structure comprised of two or more subunits. An upper limit for the number of amino acid residues corresponding to the structural gene for the enzyme would therefore be of the order of 500. Assuming the same number for yeast, then, one map unit corresponds to approximately 50 amino acid residues or, assuming a triplet code, to 150 nucleotide pairs. On this basis, exchange between mutants in the same coding unit would occur with a frequency that would correspond to less than 10^{-2} unit.

From this relationship we can estimate the lower limit of resolution imposed by the background of homoallelic reversions; recombination between two alleles can be measured only if the frequency is significantly greater than this background. We have measured the x-ray-induced homoallelic reversion frequencies of four mutants, tr_{5-2}, tr_{5-4}, tr_{5-21}, and tr_{5-36}, which appear to be repeat mutations at the same site. The mean value from nine determinations was 8×10^{-3} unit with a standard deviation of 1.5×10^{-3}. This corresponds approximately to the length of one coding unit. Thus, if the relation between map units and molecular dimensions is constant down to this level, we would expect to be able to resolve mutations in adjacent coding units. The closest pair of separable mutants we have encountered, however, is tr_{5-6} and tr_{5-18}, at a distance of 0.30 unit apart. This corresponds to about 15 amino acid residues.

The validity of the x-ray mapping method for determining the sequence of mutational sites within a gene is established by the internal consistency in our results. It is further supported by the agreement with the independently determined sequence of the ar_4 alleles. This method has several advantages over previous approaches to allelic mapping. One alternative method is to measure spontaneous mitotic reversion frequencies. These, however, can be determined accurately only by the use of a method of the type devised by Luria and Delbrück (*6*). Such methods are quite tedious, and their precision is severely limited by the nature of clonal frequency distributions (*4*). The use of x-rays circumvents these statistical limitations. Another alternative method is to measure prototroph frequencies among random spores. This, however, requires the technically difficult task, in yeast, of separating spores from unsporulated diploid cells. The x-ray method is much simpler than either of these alternatives and thereby makes extensive allelic mapping practical.

This approach does not depend on the knowledge of the mechanism of x-ray-induced reversion in heteroallelic diploid yeast. We have found, however, that the prototrophs are diploid and appear to arise by both reciprocal and nonreciprocal recombination. Double negative mutants have been recovered in some cases. A simple hypothesis that is consistent with these experiments is that x-rays produce lesions, distributed at random in the genetic material, and a lesion of the appropriate type anywhere in the region between the two mutations leads to a recombination. This hypothesis is suggested, in part, by the results of Jacob and Wollman (*7*), who found that ultraviolet light greatly increases the frequency of recombination between different mutants in crosses of the temperate bacteriophage lambda. Their studies of the kinetics of recombination after irradiation of the parental phages support the conclusion that recombination occurs with high probability at the sites of radiation-induced lesions.

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Airborne Algae: Their Abundance and Heterogeneity

Abstract. *The literature on the occurrence of airborne algae is reviewed briefly. Airborne algae were isolated into culture in both quantity and diversity. Qualitative experiments and culture techniques are discussed, as are quantitative sampling techniques and preliminary correlations of the occurrence of algae, fungi, and pollen in the air. The data disclose an important pathway for the dispersion of soil algae and support an observation that algae may be important as causal agents in inhalant allergies.*

It is well known that algae occur in and have been recovered from the atmosphere (*1*). However, these reports have not emphasized the potential abundance and variety of airborne algae because of the lack of prolonged cultivation after exposure to air. Accurate taxonomic determination of airborne algae has lagged because of failure of many investigators to realize the need for continuing studies of the morphology and physiology of these organisms in culture.

The investigations by Van Overeem (*2*) in 1937 represent pioneering efforts to recover and cultivate airborne algae. Van Overeem collected 24 air samples on six different occasions from an airplane at various altitudes over the Netherlands. Of nine different algal isolates recovered, *Chlorococcum* appeared most frequently. Samples from an altitude of 500 m provided the most abundant algal flora. Further collections at ground level, by means of an air pump, and rainwater samples revealed at least eight different species, *Chlorococcum* again being the most abundant. A Myxomycete plasmodium, moss protonemata, and fern prothallia (apparently developed in culture from spores) were also obtained by these collection methods.

Others have cultivated algae obtained from the air (*3*), but no investigators, so far as we are aware, seem to have obtained adequate data on airborne algae with respect to: (i) the heterogeneity of airborne algal genera and species; (ii) the flora of diverse geographical locations; and (iii) aerodynamically sound quantitative information. The purpose of this report is to summarize some of our recent investigations in these connections and to discuss briefly certain of their implications.

Preliminary investigations were begun in the summer of 1959 by exposing petri dishes containing sterile, solidified, inorganic nutrient medium known as "Bold's Basal Medium" (*4*). This inorganic medium did not encourage luxurious growth of heterotrophic contaminants (fungi and bacteria). Vitamin or other nutrient deficiencies later displayed by certain algal isolates in axenic culture (*5*) were apparently provided for by heterotrophs or other algae present. Table 1 lists 62 genera of algae found in the present investigation to be airborne.

To obtain qualitative information on airborne algae, we have studied collections made by the following methods: (i) sterile petri dishes (15 × 100 mm) containing agar medium were exposed to the air from 5 minutes to 12 hours at a number of stationary locations in Texas; (ii) hand-held petri dishes were exposed (3 to 5 minutes) from a moving (approximately 100 km/hr) automobile, in 14 states, and dishes were also exposed from an airplane for approximately 1 minute; and (iii) filter samples of air from 21 states supplied by stations of the National Air Sampling Network (*6*). After exposure to air, these samples were cultured under standard conditions (*7*) for 2 to 5 weeks, during which the impactions developed into macroscopically visible colonies.

Of these qualitative sampling methods, the exposures from automobiles and airplanes generally yielded the greatest quantity and diversity of algae. High-velocity winds, exerting sufficient force for effective impaction upon moist agar and immediate capture on an environment favorable for algal growth, were instrumental in the success of this method. For example, an automobile exposure in Pennsylvania on a clear, windless day yielded more than 140 algal impactions, including approximately 25 different genera. Another such exposure (10 seconds) was made recently near Austin, Texas, from an automobile moving at 60 miles per hour (96 km/hr) through a local dust cloud blowing across the highway from a plowed field. Immediate examination revealed, by direct count, less than 30 algae, but after 2 weeks' cultivation under standard conditions, more than 5000 algal impactions were recorded. Of these, 4500 were chlorophycean or xanthophycean, while approximately 500 impactions were cyanophycean. Mosaic sampling of this plate revealed a marked heterogeneity of algal genera and species, probably equal in range to what might have been obtained from an equivalent soil sample from the same site.

Exposures of petri dishes for 1 minute from a plane at 1100 m above the ground over central Texas have indi-

Table 1. Algae recovered from air and cultivated in or on "Bold's Basal Medium."

		Chlorophyta	
Borodinella	*Cylindrocystis*	*Palmellococcus*	*Scenedesmus*
Bracteacoccus	*Dictyochloris*	*Planktosphaeria*	*Spongiochloris*
*Chlamydomonas**	*Friedmannia*	*Pleurastrum*	*Spongiococcum*
Chlorella†*	*Hormidium**	*Protococcus*-like†	*Stichococcus*
Chlorococcum†*	*Hormotilopsis*	*Protosiphon*	*Tetracystis*‡*
Chlorosarcina	*Nannochloris*†*	*Psuedoulvella*-like	*Tetraspora*
Chlorosarcinopsis†*	*Neochloris†*	*Radiococcus*	*Trebouxia†*
Chlorosphaeropsis	*Oocystis*	*Radiosphaera*	*Ulothrix*
Coelastrum	*Ourococcus*	*Roya*	*Westella*
Cosmarium	*Palmella*		
		Cyanophyta	
Anabaena†	*Gloeocapsa†*	*Myxosarcina*	*Schizothrix*
Anacystis†	*Lyngbya*	*Nostoc†*	*Synechococcus*
Arthrospira	*Merismopedia*	*Oscillatoria†*	*Scytonema†*
Chroococcus-like	*Microcoleus*	*Phormidium*†*	*Tolypothrix*
Fremyella			
		Chrysophyta	
Hantschia	*Navicula*†*	*Heterococcus*†*	*Tribonema**
Melosira-like	*Botrydiopsis*†*	*Monocilia*	

* Indicates samples from airplanes in addition to ground samples. † Indicates those genera most frequently encountered. ‡ New genus, unpublished.