vol. 4. The experimental determinations of sequences by numerous workers are referenced, and the sequences are listed in the Data Section, edited by L. T. Hunt and P. J. McLaughlin. The sequences used as data for this paper are those for cytochrome c from human, horse, pig, gray whale, rabbit, gray kangaroo, chicken, king penguin, Pekin duck, pigeon, snapping turtle, Puget Sound dogfish, Pacific lamprey, fruit fly, screwworm fly, silkworm moth, tobacco hornworm moth, wheat, *Neurospora crassa*, baker's yeast, and *Candida krusei* and for cytochrome c_2 from *Rhodospirillum rubrum*. The tRNA sequences used are phenylalanine tRNA from baker's yeast, wheat, and *Escherichia coli*; tyrosine tRNA from baker's yeast, torula yeast, and *E. coli*; valine tRNA from baker's yeast, torula yeast, and *E. coli*; serine tRNA from baker's yeast and rat. Two sequences not listed in the *Atlas* are valine tRNA from *E. coli* [M. Yaniv and B. G. Barrell, *Nature* **222**, 278 (1969)] and phenylalanine tRNA from *E. coli* [B. G. Barrell and F. Sanger, *Fed. Eur. Biochem. Soc. Lett.* **3**, 275 (1969)]. Two recently determined sequences of cytochrome c from green plants support our calculations [D. Boulter, M. V. Laycock, J. A. M. Ramshaw, E. W. Thompson, in *Phytochemical Phylogeny*, J. B. Harborne, Ed. (Academic Press, London and New York, in press)]. Because cytochrome c changes more slowly than most proteins for which we can calculate mutation rates, a larger and more representative sample of protein types might give higher numbers for the evolutionary distances. However, the ratio of evolutionary distances would not be affected. For a table of mutation rates of several proteins, see P. S. McLaughlin and M. O. Dayhoff, in *Atlas of Protein Sequence and Structure 1969*, M. O. Dayhoff, Ed. (National Biomedical Research Foundation, Silver Spring, Md., 1969), vol. 4, chap. 6. For information on PAM's for proteins, see M. O. Dayhoff, R. V. Eck, C. M. Park, in *ibid.*, chap. 9.

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7. This is supported by our sequence comparisons and by biological evidence [R. H. Whittaker, *Science* **163**, 150 (1969)].
8. The variance of mutation data conforms closely to the theoretical formula for a binomial distribution, npq, where n is the length of the sequence, p is the fraction of positions which have changed, and q is the fraction of positions which have not changed. We used this formula in estimating the error.
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10. We thank Drs. L. T. Hunt, W. C. Barker, and C. M. Park for helpful discussions and assistance. Supported by NIH grant GM-08710 and NASA contract 21-003-002.

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Scanning Electron Microscopy of Developing Plant Organs

Abstract. *Shoot apices and young meristematic leaves can be examined directly with the scanning electron microscope without prior fixation or metal coating. The form of the shoot apex, cellular organization, and leaf arrangement (phyllotaxis) can be observed, perhaps as they have never been visualized before.*

A large volume of literature is available on phyllotaxis and on the form, structure, and development of shoot apices and leaves. Ordinarily, the results of these investigations are based on observations of sectioned material with the light microscope or, in some instances, of fresh material with a dissecting microscope. Permanent photographic records of entire living shoot apices are often difficult to obtain. In some instances, material is chemically fixed and stained, and the entire object is photographed in the conventional way (*1*). These preparations are often excellent, but they frequently lack a three-dimensional quality.

Einert *et al.* (*2*) have examined *Lilium* apices with the scanning electron microscope (SEM); however, their preparative procedure involved freeze-drying and exposure to acetone, which resulted in some distortion of the tissues.

It was recently shown (*3*) that surfaces of fragments of mature leaves may be examined in the wet and fresh condition with the SEM without recourse to metal coating to prevent an accumulation of surface charge. We found the same to be true for meristematic regions of the plant.

We wish to report that the SEM is an excellent instrument for examining delicate meristematic regions such as the shoot apex itself and young leaf primordia or floral appendages, at least in many species. Moreover, the SEM offers resolution and depth of field not always attainable by light microscopy. Figure 1 is representative of our research with the shoot tips of *Tropaeolum*. Older leaves were removed until the shoot apex and young leaf primordia were visible. Then the shoot tip was attached to a specimen stub with an animal-hide glue. No fixatives, de-

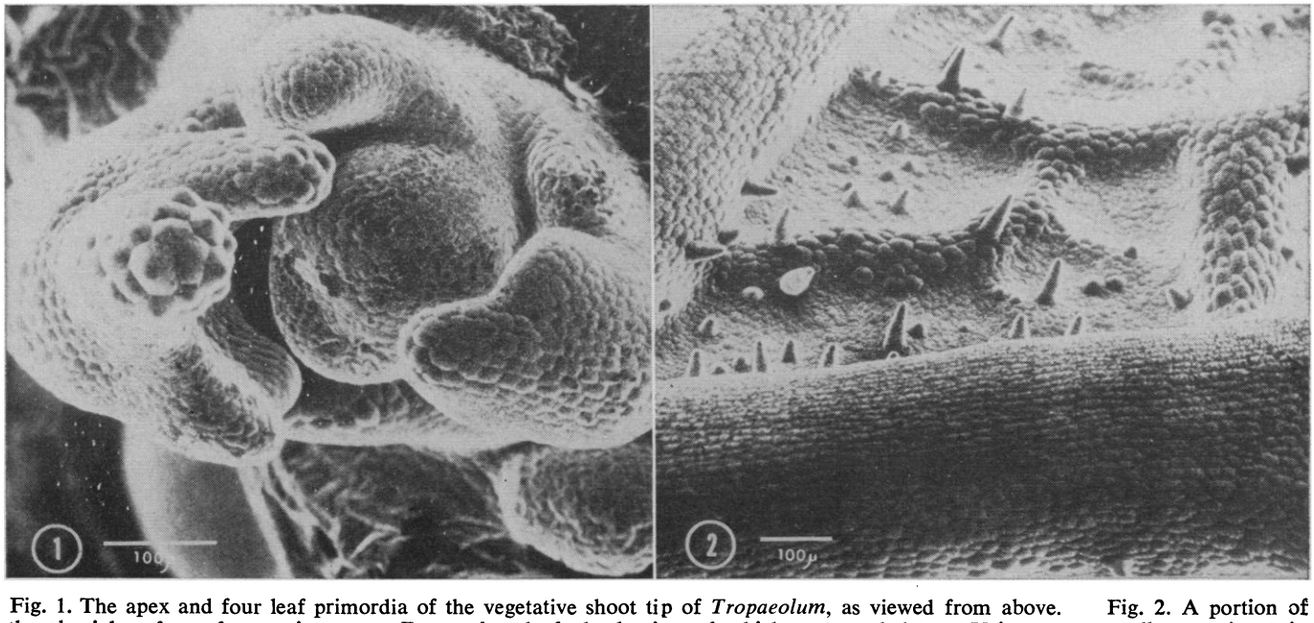

Fig. 1. The apex and four leaf primordia of the vegetative shoot tip of *Tropaeolum*, as viewed from above. Fig. 2. A portion of the abaxial surface of an entire young *Tropaeolum* leaf, the lamina of which measured 1 cm. Veins are normally prominent in leaves of *Tropaeolum*.

hydration, or metal shadowing were employed. Specimens often could be examined for 10 to 15 minutes before any detectable changes (drying out) appeared; even longer periods are possible with some other genera. Tissues near the bases of older leaves, exposed during dissection, were the first to show alterations and then these changes progressed toward the apex. Entire young leaves (Fig. 2) could be examined separately for a much longer time before alterations became apparent.

The SEM may prove to be extremely useful to plant morphogeneticists for examining the formative responses of meristematic tissues to microsurgery, to the application of growth-regulating substances, or to both. The responses of the plant tissue could be examined in considerable detail and with considerably less preparative effort than by more conventional methods.

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30 March 1970

Saccharomyces cerevisiae: A Diffusible Sex Factor

Abstract. *A hormone-like substance is secreted by α mating-type cells of heterothallic yeast strains. It induces in cells of the opposite mating type,* a, *a morphological change characteristic of the mating process. Secretion of this substance and mating ability have some common genetic determinants. In partially purified preparations, the substance has properties of an oligopeptide.*

The life cycle of heterothallic strains of the yeast *Saccharomyces cerevisiae* is characterized by a simple sexual mechanism which is under the genetic control of the mating-type locus (*1*). The two alternative allelic states of this locus are designated *a* and *α*. Haploid cells of opposite mating types conjugate to form a diploid zygote which, by mitotic division, will give rise to a clone of stable diploid cells that are heterozygous for mating type (*a*/*α*) (*2*). Under appropriate conditions such diploids undergo meiosis forming four haploid spores, two of each mating type.

The mating reaction does not require initial cell contact as the cells of mating-type *a* develop "copulatory processes" (*3*). Levi showed that this morphological response is caused by a diffusible substance when he placed cells of mating-type *a* on agar from which sexually reacting *a* and *α* cells had been removed.

We now confirm Levi's report. In addition we have partially purified a sex factor from filtrates of liquid cultures of cells of mating-type *α* and determined some of its physical, chemical, and additional biological properties. Production of this factor is controlled by the mating-type locus.

When haploid cells (*4*) of mating-type *a* are spread on a minimum agar medium (0.67 percent Difco yeast nitrogen base without amino acids, 2 percent glucose, and 2 percent agar) near a heavy streak of *α* cells, the division of *a* cells is inhibited, and they elongate to resemble those which Levi termed as having copulatory processes. The inhibition and elongation are especially pronounced when small numbers of *a* cells are streaked close to an excess of *α* cells (Fig. 1A).

Under these conditions the elongation becomes apparent after 3 to 4 hours. After 24 hours the *a* cells have formed long bizarre shapes which can reach more than ten times the length of a normal haploid cell. If the *a* cells are in excess, no elongation or inhibition is observed (Fig. 1B). Likewise, no combination of a haploid of either mating type with an *a*/*α* diploid produces this response. The inhibition and elongation, therefore, seem to be associated with the *a* phenotype in response to a diffusible factor which is secreted by the *α* cells. Identical results were observed with different, nonisogenic strains. Diploid cells which are homozygous for mating type (either *a*/*a* or *α*/*α*) behave like the corresponding haploid cells, whereas triploids, either *a*/*a*/*α* or *a*/*α*/*α*, behave like *a*/*α* diploids.

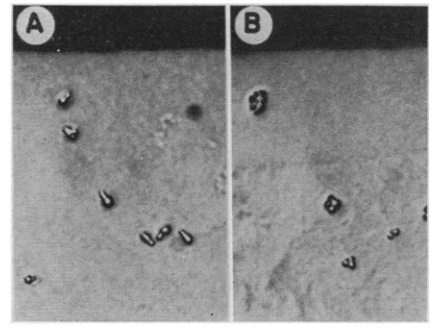

Fig. 1. (A) Cells of mating-type *a* spread near heavy streak (overnight growth) of mating-type *α* cells on minimum agar medium; (B) *α* cells near streak of *a* cells. Photographs taken after 7 hours of incubation at 30°C.

Further evidence for the correlation of the secretion of this sex factor with the *α* phenotype is provided by the properties of sterile mutants isolated from cultures of *α* cells. Haploid strains of mating-type *α*, carrying several nutritional genetic markers and the recessive allele for resistance to the arginine analog, canavanine, can^r (*5*), were exposed to ultraviolet light (374 erg/mm^2) from a germicidal lamp (General Electric). The irradiated cells (approximately 30 to 50 percent survivors) were mixed with a thousandfold excess of a mating-type *a* strain carrying complementing nutritional markers and the dominant CAN^s allele (sensitivity to canavanine). The mixture was incubated for 24 hours on YEPD agar medium (1 percent yeast extract, 2 percent Bacto-peptone, 2 percent glucose, and 2 percent agar) to allow mating. The cells were resuspended and plated on a synthetic medium that contained canavanine and the nutritional requirements of the *α* strain but lacking those of the *a* strain. Because of the dominance of the CAN^s allele, only unmated cells from the *α* strain formed colonies (*6*). Occasional diploids that had become homozygous for can^r were readily detected by their ability to grow on minimum medium. Isolates having the phenotype of the original *a* strain were tested for their ability to mate with the *a* strain by complementation.

Of 4652 clones tested, 93 were haploids that had lost the ability to mate at normal frequencies with cells of mating-type *a* and were still unable to mate with *α* cells. These sterile mutants were tested for their ability to stimulate elongation of *a* cells (Fig. 1). Sixty of them failed to effect any detectable response. The other 33 appeared to evoke varying amounts of elongation. These results strongly suggest that the ability to secrete the sex factor is under the genetic control of genes that also determine the ability of *α* cells to mate with *a* cells.

The *a*-cell response can be elicited by filtrates from liquid cultures of *α* cells. This observation has provided a method for assaying the activity, facilitating