addition, it may enable fisheries managers to identify bottom areas containing high concentrations of environmentally active and potentially toxic materials, which may require careful scrutiny, particularly for harvesting benthic or demersal organisms.

FREDERICK BOPP III
Roy F. Weston, Inc.,
West Chester, Pennsylvania 19380
ROBERT B. BIGGS
College of Marine Studies,
University of Delaware, Newark 19711

References and Notes

1. R. J. Gibbs, *Science* **180**, 71 (1973).
2. W. Krumbein and F. Graybill, *An Introduction to Statistical Models in Geology* (McGraw-Hill, New York, 1965); R. Miller and J. Kahn, *Statistical Analysis in the Geological Sciences* (Wiley, New York, 1962); J. Imbrie and E. Purdy, *Mem. Am. Assoc. Pet. Geol. 1* (1962); J. Imbrie and T. Van Andel, *Geol. Soc. Am. Bull.* **75**, 1131 (1954); J. Kovan and J. Imbrie, *Math. Geol.* **3**, 61 (1971); J. Imbrie and N. Kipp, in *Late Cenozoic Glacial Ages*, K. Turekian, Ed. (Yale Univ. Press, New Haven, Conn., 1971), p. 71.
3. Environmentally active trace metals are defined to be those cations that can be separated from the silt and clay fraction of the dried and disaggregated sediment by leaching with 10 percent hydrochloric acid at 70°C for 96 hours. Metals that are so firmly bonded to, or exchanged within, mineral grains that it is not possible to remove them under natural biochemical conditions are not considered. The laboratory extraction procedure used is designed to approximate, however crudely, the severest conceivable naturally occurring biochemical conditions without completely degrading the sediments and to reflect metals that are available for introduction to the marine food web through biological or chemical processes.
4. The raw data are contained in F. Bopp, master's (1973) and doctoral (1980) theses, University of Delaware. Because of the wide range in concentrations of the original variables, a data transformation was accomplished. The transformation, to the percentage of the maximum value for each variable, does not alter the accuracy of the factor analysis, since relative proportions and hence, locations of variance, are preserved.
5. C. Weil, *Del. Sea Grant Publ. 4-77* (1977).
6. J. Neilheisel, thesis, Georgia Institute of Technology (1973).
7. V. Klemas, M. Otley, W. Philpot, C. Wethe, R. Rogers, N. Shah, in *Proceedings of the 9th International Symposium on Remote Sensing of Environment* (Environmental Research Institute of Michigan, Ann Arbor, 1975), pp. 1289–1318.
8. Supported in part by the Sea Grant Program, grant 2-35223.

18 March 1981; revised 15 July 1981

Epifluorescence and Video Analysis of Vacuole Motility and Development in Stomatal Cells of *Allium*

Abstract. *The vacuole in stomatal cells of* Allium *undergoes major changes in shape during differentiation, switching from a globular form in new guard mother cells to a network of interconnected tubules and chambers, and then back to a globular form as guard cells mature. In addition, vacuolar network elements exhibit characteristic movements and rearrangements.*

The vacuole of plants is an organelle whose functions in cell metabolism are only beginning to be appreciated (*1*). These structures are important in photosynthesis, lysis, osmoregulation, storage, seed germination, and generation of the turgor force necessary for cell expansion. Recent advances in the isolation of vacuoles are making it possible to characterize vacuole content as well as the proteins present in the tonoplast membrane (*2*). However, despite these advances in our understanding of vacuole physiology, comparatively little is known about the development, distribution, and movement of vacuoles. Marked changes in the size and position of vacuoles occur during cell growth and differentiation. In addition, changes in vacuole morphology accompany cyclic alterations in turgor in the motor cells of leaf pulvini (*3*). Available evidence indicates that vacuoles are formed from the endoplasmic reticulum, possibly through a pathway involving GERL [Golgi-associated endoplasmic reticulum from which lysosomes form (*1, 4*)]. It has been proposed that the provacuoles that arise from this pathway constitute a network of tubular elements which coalesce to form the large central vacuole of the cell (*4*). In this report, we demonstrate vacuole motility and morphogenesis in living guard mother cells (GMC's) and guard cells of *Allium cepa* and *A. vineale*. Stomatal cells in this genus are advantageous for study because their vacuoles autofluoresce when excited with blue light (*5*). Because the vacuole stands out against the dark background of the remainder of the cell during fluorescence viewing, its organization can be more readily studied under these conditions than with phase or differential interference contrast (DIC) optics. Visualization, analysis, and archiving of fluorescence images are aided by the use of low-light-level television cameras and ancillary video equipment (*6*).

Mature guard cells in epidermal slices of *Allium* (*7*) contain large globular vacuoles located at either end of the cell and around the nucleus (Fig. 1d). The vacuole autofluoresces in the green region of the spectrum when it is excited with blue light. Emission spectra obtained with a microspectrophotometer mounted on the microscope (*8*) show a peak at 525 nm, a value similar to that previously reported (*5*).

When epidermal slices are prepared from the meristem region of cotyledons and leaves, early stages in stomatal differentiation can be examined. These observations show that vacuoles are present in the distal regions of protodermal cells undergoing asymmetric division, as well as in newly formed GMC's derived from these cells. In the latter the vacuoles are globular in shape, as judged by critical focusing with DIC optics. However, none of these vacuoles are autofluorescent. Instead, fluorescence appears in somewhat older GMC's located in more distal portions of the meristem (Fig. 1a). That the vacuole is still globular in form is evident from the fluorescence images (Fig. 1a). Fluorescence emission spectra from these young vacuoles are nearly identical to those of mature guard cells (*6*).

As the GMC's increase in size, the shape of the vacuole drastically changes. The organelle is transformed from the globular form seen earlier to a complex network or reticulum of interlinked tubules and small chambers (Fig. 1, e to j). The diameter of these elements is variable, and some as narrow as 0.1 to 0.5 μm have been seen (Fig. 1, i and j). The appearance of the network also varies from cell to cell. A GMC with a fine tubular network can exist close to another with many small spherical chambers. Careful focusing while viewing the video monitor, however, demonstrates that many if not all of the vacuole elements are interconnected.

The network is not an artifact. It is found in epidermal slices gently prepared to avoid cell damage. It is also present in GMC's located adjacent to mature guard cells that contain a globular system. Although the network is visible under fluorescence conditions, it is not evident with phase or even DIC optics. However, the reticulate nature of the vacuole was confirmed in the electron microscope with 0.5-μm-thick serial sections viewed at 100 kV (*9*).

The vacuole remains segmented in appearance during GMC division, so that each daughter guard cell receives its own network complement. The network is retained during the early stages of guard cell differentiation (Fig. 1b), but as the cells mature it is transformed back into large globules similar in form to those seen in very young GMC's and in mature guard cells. The globules are principally located at either end of the cell, but critical focusing indicates that they are interconnected by finer elements (Fig. 1c). The vacuole continues to grow in

0036-8075/81/1023-0443$01.00/0

size until it assumes the dimensions seen in the mature cell (Fig. 1d). Thus the vacuole undergoes at least two major transformations in shape during the course of guard cell formation: from globular to reticulate to globular.

In addition to these gross alterations in shape, the vacuole exhibits more subtle movements. Specifically, the spacing and distribution of network elements constantly change, and these changes are due to complex movements and rearrangements (Fig. 1, e to j). The movement is much slower than the small particle saltations and bulk cytoplasmic streaming that occur in these cells at various times. Typically, the observer becomes aware of changes in the network after 30 to 40 seconds of viewing the video monitor. Some of the movements seem to be due in part to extension of the vacuole membrane because branches can be seen to emerge from one section of the network, elongate, and sometimes fuse with another part of the network (Fig. 1, e to h). However, some movement may also be brought about by cytoskeletal interactions. Examination of thin sections as well as thick sections viewed in stereo shows that individual and small clusters of microtubules other than those that populate the cortex of these cells (*7, 10*) traverse more internal regions of the cytoplasm and closely approach the tonoplast (*6*). Although experiments on the effects of various drugs on vacuole rearrangements are incomplete, our results so far indicate that both cytochalasin B and colchicine disrupt vacuole morphology (*6, 9*).

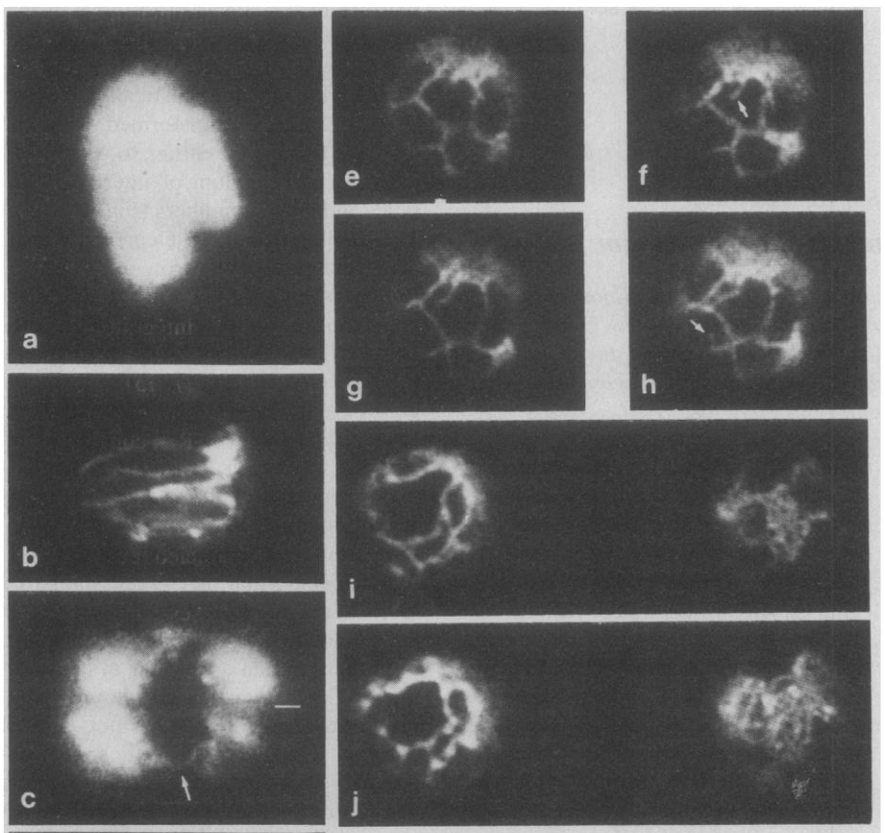

Fig. 1. Video fluorescence images of vacuoles during stages of stomatal differentiation. (a) Autofluorescent, globular vacuole in a newly formed GMC; *A. cepa*; ×2100. (b) Vacuole network in a pair of young guard cells. The common wall between cells is not evident; *A. cepa*, ×2100. (c) In maturing guard cells the network is transformed back into globules located at each end of the cell. In the tangential plane of focus (close to the leaf surface) shown here, the globules are still linked by bridging elements (arrow). The common wall between cells is marked by a horizontal dark zone (bar); *A. cepa*, ×2100. (d) Pair of mature kidney-shaped guard cells that contain globular vacuoles at each end of the cell. The stomatal pore (*P*) is located in the horizontally oriented common wall between cells. The central dark area in each cell is occupied by cytoplasm and a nucleus (*N*); *A. cepa*, ×900. (e to h) Time-lapse sequence of changes in the vacuole network of a GMC. Note the emergence and growth of tubular elements (arrows) in the upper center of (e) to (g) and the lower left of (h). (e) Zero time; (f) 28 seconds; (g) 210 seconds; (h) 341 seconds; *A. vineale*, ×1750. (i and j) Movements and rearrangements of network elements in two GMC's. Some network elements can be very narrow (0.1 to 0.5 μm), as seen in the cell at the right. (i) Zero time; (j) 200 seconds later; *A. vineale*, ×1750.

The movements do not seem to be an artifact of the processing of epidermal slices or their irradiation with high-intensity light. They were seen in cells at the initial stages of observation, as well as in those viewed after long periods of study. They were present in cells kept in the dark between observations, as well as in those maintained under constant illumination.

Our data show that the vacuole in guard cells and their precursors is a dynamic structure that undergoes programmed changes in morphology as well as smaller scale movements and rearrangements. Although networks similar to those reported here may be an initial provacuole form that coalesces to create the large central vacuole in certain cells (*4*), this does not seem to be the case in *Allium*. Clearly, the network in GMC's is derived from simple globular structures already present immediately after asymmetric division. The network is then transmitted through one cell division and is maintained in young guard cells before it is transformed back into globular form as the cells mature. Moreover, the network probably possesses at least some functions characteristic of mature vacuoles because it accumulates neutral red, potassium and chloride ions, and dicarboxylic acids (*6, 10*). Finally, other organelles including mitochondria are now known to exist as networks in other cell types (*11*). Future work will help clarify the initial ontogeny of the vacuole and the development of its fluorescence in early GMC's, as well as the mechanisms that control vacuole shape changes and motility.

BARRY A. PALEVITZ
DENNIS J. O'KANE
Department of Botany,
University of Georgia,
Athens 30602

References and Notes

1. F. Marty, D. Branton, R. A. Leigh, in *The Plant Cell*, N. E. Tolbert, Ed. (Academic Press, New York, 1980), pp. 625–658; P. Matile, *Annu. Rev. Plant Physiol.* **29**, 193 (1978).
2. G. J. Wagner and H. W. Siegelman, *Science* **190**, 1298 (1975); S. Doll, F. Rodier, J. W. Willenbrink, *Planta* **144**, 407 (1979); M. Nishimura and H. Beevers, *Nature (London)* **277**, 5695 (1979); F. Marty and D. Branton, *J. Cell Biol.* **87**, 72 (1980).
3. N. A. Campbell and R. C. Garber, *Planta* **148**, 251 (1980).
4. F. Marty, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 852 (1978); *J. Histochem. Cytochem.* **28**, 1129 (1980).
5. E. Zeiger and P. K. Hepler, *J. Cell Sci.* **37**, 1 (1979).
6. B. A. Palevitz, D. J. O'Kane, R. E. Kobres, N. V. Raikhel, *Protoplasma*, in press.
7. Epidermal slices were prepared as described in

B. A. Palevitz and P. K. Hepler [*Chromosoma* **46**, 297 (1974)] and Palevitz *et al.* (6). Slices were viewed on Reichert Zetopan microscopes equipped for epifluorescence, phase, or DIC viewing. Low-light television was performed with double- and triple-intensified video cameras (Venus Scientific Inc., Farmingdale, N.Y.) and ancillary equipment as described in (6).
8. Nanospec models SDP-2000 and 10-0112, Nanometrics Inc., Sunnyvale, Calif.
9. N. V. Raikhel, D. J. O'Kane, B. A. Palevitz, unpublished observations.
10. B. A. Palevitz and P. K. Hepler, *Planta* **132**, 71 (1976).
11. M. T. Davison and P. B. Garland, *J. Gen. Microbiol.* **98**, 147 (1977); B. Grobe and C. G. Arnold, *Protoplasma* **86**, 291 (1975); M. Pellegrini, *J. Cell Sci.* **46**, 313 (1980).
12. Supported by National Science Foundation grant PCM81-04470 to B.A.P. and Public Health Service national research service award to D.J.O.

1 July 1981; revised 11 August 1981

Complete Nucleotide Sequence and Organization of the Moloney Murine Sarcoma Virus Genome

Abstract. *The complete nucleotide sequence of a mammalian transforming retrovirus, Moloney murine sarcoma virus, has been determined. MSV, a recombinant virus derived of helper viral and cellular sequences, possesses termini resembling prokaryotic transposable elements. The viral genome has the coding capacity for the Moloney murine leukemia virus* gag *gene product and contains large deletions in* pol *and* env *genes. A large open reading frame encompassing its cell-derived sequences codes for its putative transforming protein. The nature of some of the important domains in the viral genome has been established, and their structure is discussed in relation to their function.*

Type C RNA viruses (retroviruses) represent a class of genetic elements capable of neoplastic transformation in their natural host. These viruses can be grouped into two broad classes on the basis of their biological activities. The leukemia or leukosis viruses are replication-competent, cause leukemia in susceptible hosts, but do not transform cells in tissue culture. In contrast, the sarcoma viruses, which are usually replication-defective, cause neoplastic transformation of fibroblasts in vitro and produce solid tumors in vivo.

Moloney murine sarcoma virus (MSV) is a representative of the class of replication-defective sarcoma viruses. This virus arose by recombination of the nondefective Moloney murine leukemia virus (MuLV) and cellular sequences present within the normal mouse genome (*1–3*). These latter sequences are essential for viral transforming activity (*3–5*). According to recent convention (*6*) the cell-derived sequences of MSV are designated v-*mos* and those of the cell are designated c-*mos*. The development of molecular cloning and DNA sequencing techniques has made the detailed analysis of the virus genome structure possible. We present in this report the first complete sequence analysis of a transforming retroviral genome.

Figure 1 provides a detailed restriction map of the 5.8-kbp (kilobase pair) MSV genomic DNA cloned in bacteriophage λ (*2*) as well as our sequencing strategy. Sequence analysis was performed according to the procedures of Maxam and Gilbert (*7*). The entire MSV nucleotide sequence is presented as it occurs in the linear proviral genome in Fig. 2.

An important structural feature of the MSV genome is the occurrence of two large terminal repeats of 585 bases (LTR's) at both 5′ and 3′ ends of the proviral genome. These LTR's bear striking similarities to the terminal repeats of prokaryotic transposable elements (*8, 9*). Previous studies have provided sequence data for this region of the genome (*10–15*). The salient features are summarized below.

1) *Inverted terminal repeats.* An inverted repeat of 11 nucleotides, 5′-TGAAAGACCCC-3′ (T, thymine; G, guanine; A, adenine; C. cytosine), appeared at the termini of each LTR at positions 1 to 11, 575 to 585, 5244 to 5254, and 5818 to 5828. Prokaryotic transposable elements are also flanked by such inverted repeats and are capable of translocation to different positions on the chromosome or to another replicon in the cell (*8, 9*). Like these bacterial elements, retroviruses integrate into the host DNA in a linear orientation with defined endpoints (*10, 11, 15*).

2) *Transcription initiation and termination signals.* The LTR is composed of a track of about 440 nucleotides derived from the 3′ end of the viral RNA, directly followed to the right by a stretch of approximately 145 nucleotides derived from the 5′ end of the viral genome (*16*). This stretch of 585 bases contained sig-

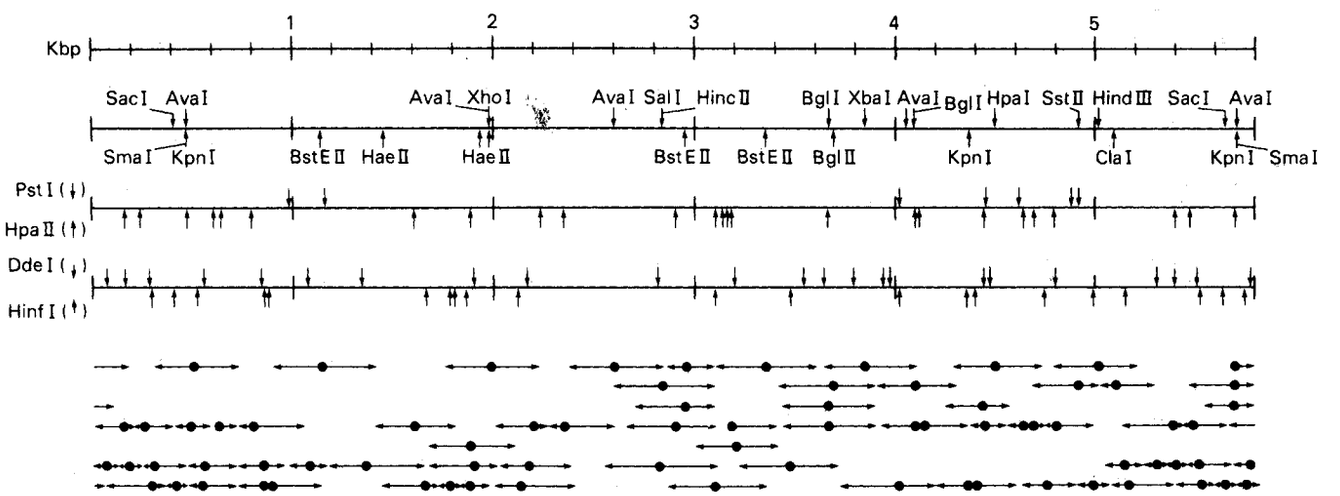

Fig. 1. Restriction enzyme map and strategy for sequencing of the MSV genome. The genome was sequenced with the use of the restriction sites indicated on the diagrammatic map. The 5′ ends were labeled with [γ-^{32}P]ATP and T_4 polynucleotide kinase (*7*). The 3′ ends were labeled with [^{32}P]cordycepin phosphate and terminal transferase (*48*). The labeled end of each fragment is indicated by the filled circle and the extent and direction of sequencing are indicated by the arrows.

0036-8075/81/1023-0445$01.00/0