THREE DIMENSIONAL STRUCTURE OF THE CELL CENTER REVEALED BY COMPUTER GRAPHICS METHODOLOGY

NAHUM D. GERSHON,* KEITH R. PORTER,‡ AND MARK A. MCNIVEN‡
*Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, and Physical Science Laboratory, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland 20205; and ‡Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228

The cell center in many cells comprises two centrioles and a complex of dense bodies (e.g., see reference 1). These function as initiating sites for the assembly of microtubules. Thus the cell center complex influences the disposition and orientation of microtubules and appears to be involved in cell form determination.

We have used the techniques of computer graphics to reconstruct in three dimensions the basic form of this apparatus in pigment cells and to document any changes it may undergo as the cell aggregates its pigment along tracks provided by the microtubules. It was found by three-dimensional reconstruction that the dense bodies are arranged in a number (in the range of 3–7) of planar areas, or plates, separated by ~100 nm. The complex exists as a stack of these planar units with the centrioles in a depression near the center of the axis of this stack. A shift in the position of dense bodies peripheral to the complex accompanies the dispersion of pigment.

To study the cell center we chose to examine the erythrophores of squirrel fish (*Holocentrus ascensionis*) in which the dense bodies are clearly seen in osmium-stained electron microscope preparations (2). The cell center complex is much larger than the thickness of the sections viewed by the high-voltage electron microscope (~200 nm). Hence one must take data from a series of thick sections. The difficulty in analyzing cellular components spanning many sections is that it is hard for our visual-mental machinery to grasp the three-dimensional structure of the integrated object by looking on the sections one at a time. Thus we had to develop a three-dimensional reconstruction method using a computer with color graphics capabilities. The resulting three-dimensional object can be presented on the computer screen and viewed from different directions.

METHODS

The input for the three-dimensional reconstruction was from micrographs generated by the high-voltage electron microscope in University of Colorado at Boulder, CO (JEOL, Japan). The contours of the dense bodies and the centrioles were manually digitized using a Summagraphics ID digitizer. The data were then processed by a Cromemco System 2 computer (with 68000-based processor) together with MicroAngelo color boards (Scion Corp., Vienna, VA). The computer aligned the serial sections using fiduciary marks that were identified in each section using a Ladd stereoscope (Model SB180/183). In most instances cross sections of collagen filaments were used; these repeated in several sections. The aligned sections were then used to form the reconstructed image, which was plotted in color on the monitor in two or three dimensions. The resulting image could be then rotated on the screen and thus viewed from different directions.

RESULTS AND DISCUSSION

The computer system just described was used to reconstruct the cell center of the erythrophores of *Holocentrus*. One of the micrographs used in the reconstruction is

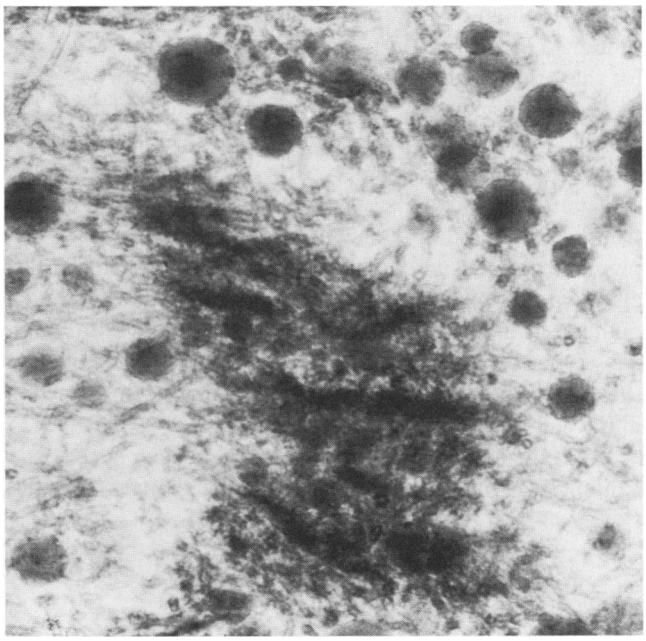

FIGURE 1 The microtubule organizing centers in the erythrophore of *Holocentrus* in the dispersed state. This is one of the sections that was used to reconstruct the cell center. The dark and usually elongated entities are the dense bodies from which microtubules grow.

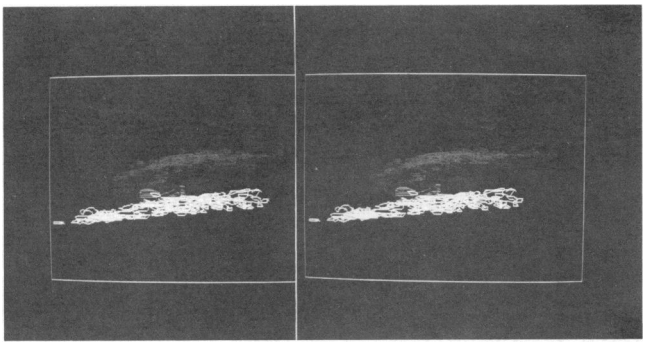

FIGURE 2 The cell center in a cell in the aggregated state, viewed from the side. The microtubule organizing centers are organized in plates colored in orange, red, and white. The centrioles are plotted in green and are located between two of the plates. This image is a reconstruction from 20 sections. Please refer to the color figure section at the back of this book.

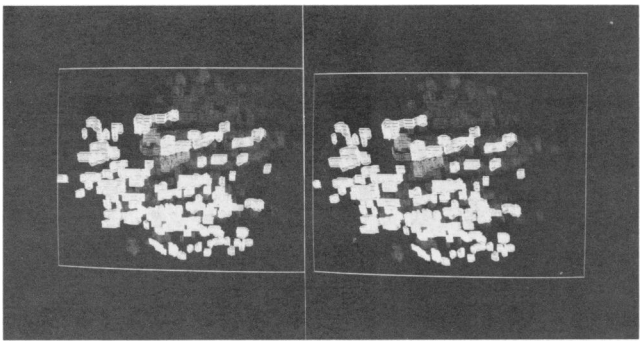

FIGURE 3 A tilted view of the same cell center shown in Fig. 2. Please refer to the color figure section at the back of this book.

presented in Fig. 1. The resulting images are given in Figs. 2 and 3. The main part of the cell center in these erythrophores is seen to be organized largely in parallel planar plates. The centrioles, which are perpendicular to each other, are situated in the top portion of the dense body complex. The distribution of the dense bodies in each plate appears to be reticular. These features of the organization of the cell center were also found in the dispersed state; the only difference was that the dense bodies in the periphery of this complex were found to be more widely scattered.

This highly organized structure is basically stable during the aggregation and dispersion of the pigment granules. It is not known what holds the complex of the dense bodies and the centrioles together. The microtubular network with or without other proteins, like microtubule-associated proteins (MAP), could provide the restricting framework for this organization.

The plates, which consist of the dense bodies, are parallel to the flat basal surface of the cell. Thus there is a correlation of the cell shape with the organization of the cell center. It seems likely that microtubules that emanate from a given plate influence the tracking of the motion of pigment granules in one horizontal region of the cell. The aggregation could be a multilevel process in which each pigment granule is assigned to a particular horizontal level. This type of arrangement seems to be more efficient than the one in which all the granules move to one central region in the cell.

From the micrographs it is evident that each dense body can nucleate more than one microtubule. Thus the organization of the dense bodies comprises the location and the orientation of each body in space plus the internal organization of each nucleation site within each body. From these considerations it is clear that the organizing mechanism of the cell center is quite complicated. To study it involves determination of the location and orientation of the dense bodies and their internal structure.

SUMMARY

The three-dimensional reconstruction studies presented here indicate that the cell center in the erythrophores of *Holocentrus* is highly organized. The main features of this organization do not change during the massive changes that the cell undergoes during the aggregation and dispersion of the pigment granules. The structure composed of plates consisting of individual dense bodies is parallel to the basal surface of the cell, thus creating a multilevel or stacklike configuration. This arrangement might render the aggregation-dispersion process more efficient than otherwise possible. From these findings and considerations it seems that the cell center is an organelle where information about the three-dimensional structure of the cell is located.

Received for publication 6 May 1985.

REFERENCES

1. Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. 1983. Molecular Biology of the Cell. Garland Publishing, Inc., New York and London.
2. Porter, K. R., M. Beckerle, and M. McNiven. 1983. The cytoplasmic matrix. *In* Spatial Organization of Eukaryotic Cells - A Symposium in Honor of K. R. Porter. J. R. McIntosh, editor. Alan R. Liss, Inc., New York. 259–302.