NEW STRATEGIES FOR DETERMINING RIBOSOMAL SUBUNIT STRUCTURE FROM ELECTRON MICROGRAPHS BY SINGLE PARTICLE AVERAGING METHODS

J. FRANK,* A. VERSCHOOR,* T. WAGENKNECHT,* M. RADERMACHER,* M. ROSENTHAL,* AND M. BOUBLIK‡

**Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201; and ‡ Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110*

Projections of ribosome particles can be routinely obtained with high statistical definition, by application of single particle alignment and averaging techniques to electron microscopy images (1). [1,2] By combining projections corresponding to different viewing directions, three-dimensional structural information may be retrieved (2, 3).

Uranyl salts commonly used for staining in conventional electron microscopy limit the structural investigation of ribosomes and their subunits in four ways: (*a*) positive staining effects may produce ambiguities in the interpretation of projections or of three-dimensional data derived from these; (*b*) little information is obtained on the interior of the nucleoprotein mass; (*c*) the inherent granularity of the stain limits the resolution with which the particle boundary can be reliably determined; and (*d*) the low pH at which these stains are usually used and their ionic nature sometimes affect their native structure. We have therefore begun to explore alternative specimen preparation techniques utilizing different stains or embedding media. However, when such techniques are used, some of the correlation detection algorithms currently used for alignment may no longer be applicable (4). The exploration of new preparative techniques must therefore be accompanied by a search for more powerful mathematical tools.

[1]Verschoor, A., J. Frank, and M. Boublik. 1985. Investigation of the 50S ribosomal subunits by electron microscopy and image analysis. Unpublished results.

[2]Verschoor, A., J. Frank, T. Wagenknecht, and M. Boublik. 1985. Computer averaged views of the 70S monosome from *Escherichia coli*. Unpublished results.

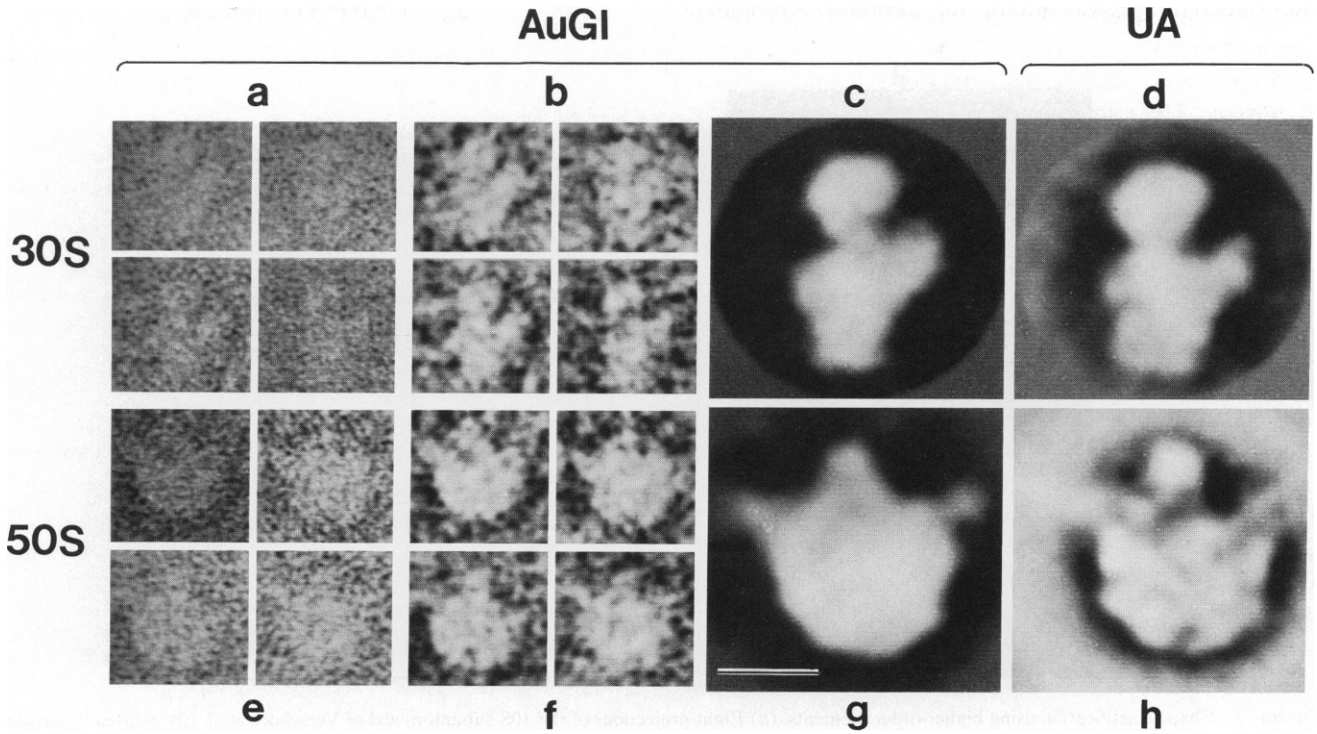

FIGURE 1 Images of 30S and 50S ribosomal subunits of *E. coli*, stained with aurothioglucose (*a–c*, *e–g*) and uranyl acetate (*d*, *h*). (*a*, *e*) example of images scanned at 0.5 nm sampling resolution; (*b*, *f*) images shown in *a*, *e* lowpass filtered to 3 nm limiting resolution; (*c*, *g*) averages obtained with N = 51 and 27 (scale bar 10 nm); (*d*, *h*) averages obtained with N = 6 and 30.

ALTERNATIVES TO URANYL SALT STAINING

Aurothioglucose (AuGl) has been previously suggested as a medium that preserves the three-dimensional shape of proteins and provides uniform nonspecific contrast (5). In addition, the density of the stain may be reduced in a controlled manner by mixing AuGl with glucose, thereby allowing the protein and ribonucleic acid components of ribosomes to be selectively contrasted. Quantitative comparison of uranyl- and AuGl-stained ribosomes should reveal regions of positive staining by uranyl ions, presumably corresponding to regions where rRNA is exposed on the surface of the ribosome. As a first step, we have tried to analyze electron micrographs of 30S and 50S ribosomal subunits from *Escherichia coli* stained with AuGl.

The contrast of the images (Fig. 1 *a, e*) was, as expected, considerably lower than that observed with uranyl stains, but it improved markedly by low-pass filtration (Fig. 1 *b, f*). Nevertheless, even filtered images of the 50S subunit assigned to the generally recognizable "crown" orientation proved difficult to align by correlation methods (success rate ~30%). Alignment of 30S subunits was usually possible, owing mainly to their elongate shape, but identification of the many views arising from rotations about the long axes of the particles was a problem. We used correspondence analysis (6, 7) to select a homogeneous image set of aligned 30S subunits in the intermediate view (Lake's terminology [8]). Images of the 50S subunits in the crown orientation were visually selected; ~25% of the original images was suitable for averaging. Averages of the two subunits are shown in Fig. 1 *c, g* along with corresponding averages (Fig. 1 *d, h*) obtained previously using uranyl acetate (2).[1] At the present stage of our studies, the averages (Fig. 1 *d, h*) obtained with AuGl are in general agreement with those obtained using uranyl stains. However, in the case of the 50S subunit, a pronounced difference in staining occurs in the "head" region that we believe is due to the interactions between uranyl acetate and exposed RNA.

Preliminary studies of frozen-hydrated 50S subunits (in collaboration with R. Grant and W. Chiu, University of Arizona) indicate that frozen-hydrated specimens will be even more difficult to analyze than isolated particles by existing methods. The need for new methods of processing images of this type is apparent.

CLASSIFICATION OF PROJECTIONS BY MOMENT-INVARIANTS

The difficulties posed by spurious and unreliable correlation peaks are compounded when ice embedding is being used without support for maximum contrast (9), because particles may assume random orientations without restrictions. Classification techniques requiring prior alignment (6, 7) and sophisticated multireference alignment methods (10) may not work in this situation. What is needed instead is a general method of shape classification that is invariant under rotation and translation in the plane of the micrograph. Hawkes (11) suggested the use of higher moments, defined as

$$\mu_{pq} = \int\int x^p y^q f(x, y)\, dx, dy,$$

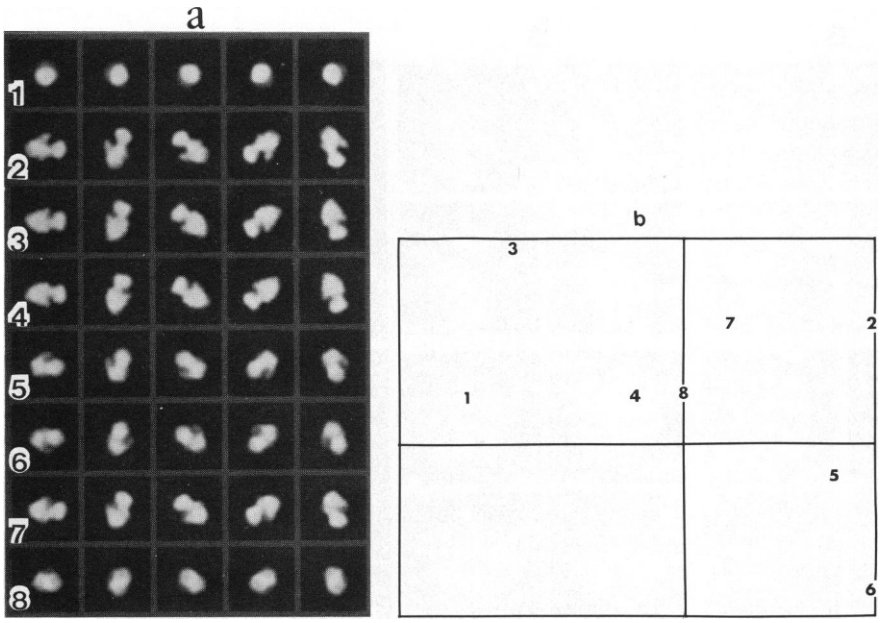

FIGURE 2 Shape classification using higher-order moments. (*a*) Eight projections of the 30S subunit model of Verschoor et al. (2), rotated by various angles in the image plane. ϕ and θ values: 1: 0°, 0°; 2: 0°, 90°; 3: 90°, 90°; 4: −90°, 90°; 5: 0°, 45°; 6: 180°, 45°; 7: 45°, 60°; 8: −45°, 30°. (*b*) Correspondence analysis map (1 vs. 2) of the invariant-profiles $\phi_1 \ldots \phi_7$ given by Hawkes (11). The first factor axis (vertical) accounts for 57% of the total interprofile variance, and reflects the degree of complexity of the shapes; the third factor axis (horizontal; 2.5%) is related to the degree of ellipticity. (The clusters formed by each projection set are so small that only a single symbol was used for each group of five rotated versions).

where $f(x, y)$ is the density distribution in the image; x, y are coordinates relative to its center of gravity; and p, q are integers; $p + q$ is the order of the moment.

By combining these moments, certain invariants can be formed which may be used to describe the image with increasing accuracy as the order of moments included increases (14). Instead of the images themselves, the profiles formed by its moment-invariants ϕ_i, $i = 1 \ldots n$ may therefore be used for the identification of projections in a micrograph of randomly oriented particles.

To explore the feasibility of this approach, we have computed general (ϕ,θ) projections of the 30S ribosomal subunit previously reconstructed by Verschoor et al. (2), and rotated these in the image plane by various azimuths. The set of projections so created (Fig. 2 *a*) simulates the appearance of some of the particle views in the micrograph, assuming that the three-dimensional model is correct and provided that no deformations of the particle take place. The seven-moment invariants $\phi_1 \ldots \phi_7$ associated with $p + q = 3$ that were given by Hawkes (11) were computed for each of the 40 ($= 8 \times 5$ azimuths) projections. Because of quantization errors due to image rotation or due to sampling of differently oriented images, the calculated ϕ values undergo fluctuations (see reference 12). At this stage of the investigation we would like to establish whether or not different projections of an asymmetric particle such as the ribosomal subunit give rise to recognizably different invariant-profiles, and also whether or not the quantization errors would tend to obscure divisions among classes found.

The invariant-profiles formed by $\phi_1 \ldots \phi_7$ were subjected to correspondence analysis (6, 7). What is apparent from the map of factor 1 vs. 3 (Fig. 2 *b*) is that the projections are recognized as different, irrespective of their in-plane orientation. (Similar grouping occurs on the map of factor 1 vs. 2 but with some ambiguities.) The model computation suggests that it may be possible, at least crudely, to identify molecular projections in noisy micrographs.

This material is based upon work supported by grant 1R01 GM 29169 from the National Institutes of Health.

Received for publication 8 May 1985.

REFERENCES

1. Frank, J., A. Verschoor, and M. Boublik. 1981. Computer averaging of electron micrographs of 40S ribosomal subunits. *Science (Wash. DC)*. 214:1353–1355.
2. Verschoor, A., J. Frank, M. Radermacher, T. Wagenknecht, and M. Boublik. 1984. Three-dimensional reconstruction of the 30S ribosomal subunit from randomly oriented particles. *J. Mol. Biol.* 178:677–698.
3. Van Heel, M. 1983. Three-dimensional reconstruction of the 30S *E. coli* ribosomal subunits. *In* Proc. Electron Microscopy Soc. of America, 41st. G. W. Bailey, editor. San Francisco Press, San Francisco. 460–461.
4. Saxton, W., and J. Frank. Motif detection in quantum noise-limited electron micrographs by cross-correlation. *Ultramicroscopy*. 2:219–227.
5. Kühlbrandt, W., and P. N. T. Unwin. 1982. Distribution of RNA and protein in crystalline eukaryotic ribosomes. *J. Mol. Biol.* 156:431–448.
6. Van Heel, M., and J. Frank. 1981. Use of multivariate statistics in analyzing the images of biological macromolecules. *Ultramicroscopy*. 6:187–194.
7. Frank, J., and M. van Heel. 1982. Correspondence analysis of aligned images of biological particles. *J. Mol. Biol.* 161:107–137.
8. Lake, J. 1976. Ribosome structure determined by electron microscopy of *Escherichia coli* small subunits, large subunits, and monomeric ribosomes. *J. Mol. Biol.* 105:131–159.
9. Adrian, M., J. Dubochet, J. Lepault, and A. W. McDowall. 1984. Cryo-electron microscopy of viruses. *Nature (Lond.)*. 308:32–36.
10. Van Heel, M., J.-P. Bretaudiere, and J. Frank. 1982. Classification and multireference alignment of images of macromolecules. *In* Electron Microscopy 1982 - Proc. Intern. Congr. El. Microsc., 10th, Deutsche Gesellschaft für Elektronenmikroskopie e.v. Frankfurt. 1:563–564.
11. Hawkes, P. W. 1980. Some unsolved problems and promising methods in electron image processing. *In* Electron Microscopy at Molecular Dimensions. W. Baumeister and W. Vogell, editors. Springer Verlag, Berlin. 288–299.
12. Teague, M. R. 1980. Image analysis via the general theory of moments. *J. Opt. Soc. Am.* 70:920–930.

SYNTHETIC OLIGODEOXYNUCLEOTIDES AS PROBES OF RNA CONFORMATION WITHIN THE RIBOSOME

D. G. GLITZ, H. M. OLSON, AND L. S. LASATER
Department of Biological Chemistry, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

The prokaryotic ribosome is composed of over fifty distinct proteins plus three different RNA molecules in two dissimilar subunits. Electron microscopy has been of great value in the determination of ribosome and ribosomal subunit structure, and antibodies directed against specific ribosomal proteins have allowed placement of several proteins within the structure of each subparticle. A reasonable consensus on protein localizations has begun to emerge (1).

Much less is known about placement of the ribosomal RNA. About two-thirds of the mass of the *Escherichia coli*