Direct Demonstration of Eight-fold Symmetry in Nuclear Pores*

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Summary. Pictures, in front view, are presented of the nuclear pores from the oocytes of the newt Taricha granulosa. Negative staining is used. It is directly visible, on a substantial proportion of the pores, that the number of subunits in the annulus is 8. This conclusion had been reached earlier by other writers, who had used the rotation technique to ascertain the radial symmetry. The rotation technique is known to be very unreliable, though on this occasion had produced the correct result. A fibrous mesh network, connecting the subunits of separate pores is described.

Key words: Nuclear pore — Amphibian oocyte — 8-fold symmetry — Fibrous network — Electron microscopy — Negative staining.

Introduction

The ultrastructure of the nuclear pore has been the subject of many recent investigations. It will be unnecessary to give any general review as this has been done by several authors, whose papers contain extensive bibliographies (e.g. Franke, 1970; LaCour and Wells, 1972; Mentré, 1969; Vivier, 1967; Wunderlich and Speth, 1972; Yoo and Bayley, 1967).

These studies leave no doubt that the nuclear pore shows a very remarkable degree of similarity in a wide range of organisms, going from mammals to protozoa and plants. The present paper is devoted to establishing the eight-fold symmetry of the subunits by direct inspection, without any ambiguity. A fibrous network connecting the pores will also be described.

Review of Literature

Several authors (among them: Kessel, 1969; Rehbun, 1956; Speth and Wunderlich, 1970; Watson, 1959) and those listed in the first paragraph of the introduction have noted that, in front view, the annulus of the pore seems discontinuous. Watson (1959) first remarked that the number of elements in the annulus seemed to be close to eight, and many later writers report the same observation. The only decisive pictures we know are those of Daniels et al. (1969) in the amoeba Pelomyxa. In most publications no definite symmetry is even suggested; in others, discontinuities in the annulus are marked with eight radial arrows or lines, in cases considered suggestive (e.g. Kessel, 1969; Monroe et al., 1967; Wunderlich and Speth, 1972). Likewise, Wolstenholme (1966) remarks that some of the subunits lie at 45°, and that the symmetry is therefore likely to be ×8. In an attempt to

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obtain a decision as to the symmetry, Franke (1966) applied the Markham et al. (1963) rotation technique for analysing radially symmetrical images. Several other authors have since used the same procedure on images of the nuclear pore, in a considerable range of material (Abelson and Smith, 1968; Bajer and Molé-Bajer, 1969; de Zoeten and Gaard, 1969; Fisher and Cooper, 1967; Franke, 1967, 1970; Franke and Kartenbeck, 1969; Franke and Seheer, 1970; Gall, 1967).

The rotation technique has been discussed and criticized several times (Agrawal et al., 1965; Norman, 1966; Friedman, 1970; Finch et al., 1966; Crowther and Amos, 1971) and it is clear that it can produce artifacts in several ways. On the one hand, submultiples of the true frequency can generate an apparently striking reinforcement, as Friedman (1970) demonstrates both on artificial models and actual pictures. A more deceptive artifact, as Crowther and Amos (1971) explain at length is that any slight asymmetry, such as might result from tilt or bilateral deformation, results in strong harmonics close to the true one. These authors have evolved a much more elaborate form of analysis, in which an optical density scan is subjected to a Fourier transform by means of a computer program, and a rotational power spectrum is finally generated. The strongest symmetry present can then be read from a plot of the power spectrum and compared to any other symmetries. In addition, the best point of origin can be ascertained by objective criteria, equivalent to the center of rotation analysis. Examination by these methods of images from actual preparations clearly demonstrates that what appears to be the strongest reinforcement to the eye by no means always corresponds to the strongest symmetry shown by the power spectrum. A direct demonstration of this artifact is that the chief illustration of their method used by Markham et al. (1963), TMV protein, gives apparent maximum optical reinforcement for 16-fold symmetry, and this 16-fold symmetry is selected by the authors. But it can be demonstrated that the true symmetry of TMV protein is 17-fold (Finch et al., 1966; Crowther and Amos, 1971). The complications are compounded by difficulties of inductive logic, which arise because there is freedom to select a good image for analysis, the criteria of goodness not being independent of the particular symmetry one hopes to find. There is in addition freedom in selecting the best rotation center, a slight displacement of which generated extraneous harmonics. Even the rotational harmonic analysis of Crowther and Amos (1971) is only applicable if the symmetry as detected on the power spectrum plot is obvious, because a statistical technique for judging the significance of a doubtful symmetry is lacking.

Thus it seems clear that the Markham et al. technique is intrinsically incapable of establishing a symmetry which is not already obvious in the unrotated image; in other words, it can only produce a reliable answer when that answer is not needed.

The authors cited earlier, who applied the rotation technique, usually report that they got their best reinforcement at $\times 8$ symmetry. In several cases it is not reported how many pictures were tested, or what symmetries besides $\times 8$ were explored. In the cases which are described most fully, the results are by no means unambiguous. Gall investigated three organisms: about fifty rotation tests were made, and there are illustrations of four tests from Triturus and one each from Rana and Henricia. While all four rotations from Triturus show reinforcement for $\times 8$, three show reinforcement for $\times 7$ as well, and two for $\times 9$. The Rana test