Characterization of the Nuclear Lamina in an Insect by Differential Staining

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The nuclear envelope of the larval fat body in the blowfly *Calliphora vicina* reveals a conspicuous nuclear lamina. It appears homogeneous, fibrous or granular depending upon the fixing and staining methods. The best relative contrast for the nuclear lamina and the nuclear pore complex is obtained with a phosphotungstic acid staining procedure designed for proteins.

In the last few years, the nuclear envelope has found increasing interest with regard to its biochemistry and function. Briefly, the nuclear envelope consists of an outer and an inner nuclear membrane, separated by the perinuclear space, and interrupted by the nuclear pores. An additional non-membranous layer adjacent to the inner nuclear membrane, also pierced by the nuclear pores, has been found in many classes of eucaryotes (for review see [1 – 5]). The third layer forms a honeycomb-like structure in some species of protozoa [6, 7] and in some cell types of *Hirudo medicinalis* [8]. It is present as a 20 – 80 nm thick layer in several classes of vertebrates including man [1 – 5, 9 – 12]. This layer was termed the fibrous lamina [9], zonula nucleum limitans [10], dense lamelle [11] or lamina densa [4]. Now the term nuclear lamina (NL) or peripheral nuclear lamina is preferred (for the terminology of the nuclear components see [13]). The fine structure of the NL has been described as fibrous [9, 10, 12], granular [10 – 12], or homogeneous [11] when examined by transmission electron microscopy. A fibrous [14] or granular layer [15] has also been demonstrated by scanning electron microscopy. The NL and the nuclear pore complex consist mainly of proteins; carbohydrates or nucleic acids have not been found in significant amounts [2 – 5, 16 – 18]. More recently, differences in protein patterns related to species, cell type, and cell cycle have been described (*e.g.* [19, 20]).

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In insects, the characterization of the NL has been rather neglected although a third layer in the nuclear envelope of the salivary gland of *Drosophila hydei* has been reported in 1963 [21] and mentioned for *D. melanogaster* [5]. In the blowfly *Calliphora vicina* a more or less thick NL is observed in all tissues which we have examined to date. The very distinct NL in the intact tissue and in isolated nuclei of the larval epidermis has been described as "zonula nucleum limitans" in an earlier paper [22].

In the present report, a method for the identification of the NL is compared to some other treatments. For these studies, the fat body of the third instar larvae (7 days old) of *C. vicina* was used. All

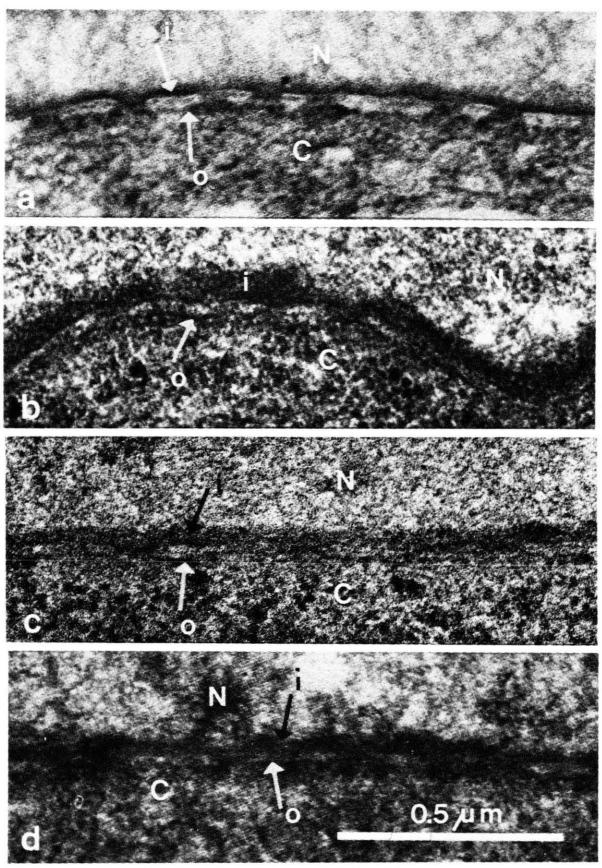

Fig. 1. Electron micrographs of the nuclear lamina in fat body cells of larval *Calliphora vicina* after different treatments of fixation and staining. × 60,000. a) Phosphotungstic acid for proteins, b) glutaraldehyde and osmium tetroxide, c) dichromate-Os-PTA-uranyl acetate, d) ruthenium red (details in the text). N nucleoplasm, C cytoplasm, o outer nuclear membrane, i inner nuclear membrane with the adjacent nuclear lamina.

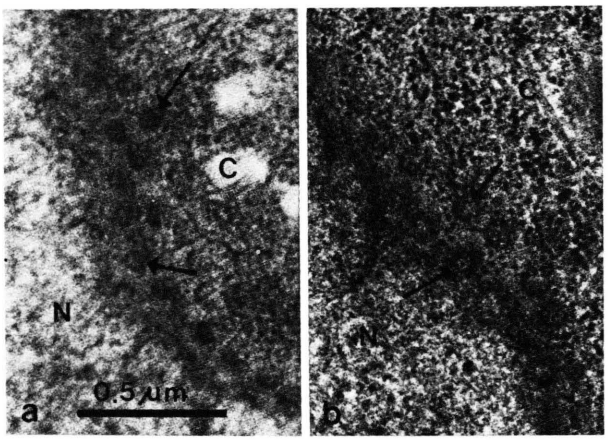

Fig. 2. Electron micrographs of tangential sections of the nuclear envelope, showing the nuclear pores (arrows). ×40,000. a) Phosphotungstic acid for proteins, b) glutaraldehyde and osmium tetroxide.

methods related to the rearing of the animals and general processing of the tissue for electron microscopy have been described previously [23]. Depending on fixation and staining the following observations on the structure of the NL have been made:

a) With glutaraldehyde (GL) fixation and phosphotungstic acid (PTA) staining of proteins [24], the NL is seen as a 25 – 35 nm thick homogenous layer of high electron density (Fig. 1 a). The perinuclear space contains a somewhat interrupted dense line. The pore walls are also densely stained (Fig. 2 a). In the nucleoplasm, a network of fibres is visible. Membranes remain unstained. Carbohydrates (*e.g.* glycogen) and free DNA do not react.

b) After "conventional" fixation with GL in cacodylate buffer and osmium tetroxide (Os) in veronal acetate buffer with sucrose [25], the NL appears as a 50 – 70 nm thick fibrous and granular structure in epon sections poststained with lead citrate (Pb; Fig. 1 b). The nuclear pores reveal usual features after poststaining with uranyl acetate (U) and Pb (Fig. 2 b).

c) Fixation with GL, followed by potassium dichromate and Os, impregnation with alcoholic PTA and U [26], and poststaining of the sections with U and Pb, exhibits a rather diffuse, very fine granular and somewhat fibrous structure of the NL which appears 30 – 50 nm thick (Fig. 1 c). With this fixation, a better preservation of proteins and nucleic acids may be expected.

d) Ruthenium red (RR) treatment at pH 7.4 [27] reveals a more diffuse structure with some fibres of the NL and a thickness of 30 – 50 nm in unstained sections (Fig. 1 d). After poststaining of the sections with Pb, the structure is more granular (not shown). RR has a certain specifity for acidic carbohydrates and lipids, *e.g.* acidic phospholipids [28].

The NL is clearly visible using any of the above methods. However, the NL shows the best relative contrast with PTA (a), since it ist not masked by other cell components. Thus the PTA method used here may be a helpful and suitable tool for the recognition of the NL. The differences in the structure depending on fixation and staining give rise to the assumption that a variety of compounds, besides proteins, could possibly be involved in the architecture of the NL.

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