Localised Uptake of $^{63}$Nickel into Dinoflagellate Chromosomes: An Autoradiographic Study

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Summary

The uptake of $^{63}$Ni into cells of the binucleate dinoflagellate Glenodinium foliaceum was investigated using insoluble compound light and electron microscope autoradiography. Cells labelled over a period of 2 hours showed active uptake throughout the whole population, with an increase in mean cell grain count when the labelling period was extended to 4 hours and 24 hours. The mean grain count did not vary with type of fixation (glutaraldehyde, paraformaldehyde or acetic alcohol) suggesting that retention of $^{63}$Ni is not a specific fixation-binding artefact. At light microscope level, silver grains were not localised to any major cell component, but with the greater resolution of electron microscope autoradiography, a high degree of localisation was demonstrated in the typical dinoflagellate (dinocaryotic) nucleus — which contained about 83% of the cell label (cytoplasm 16%, supernumerary nucleus 1%). Silver grain distribution within the dinocaryotic nucleus was consistent with some degree of localisation to the condensed chromatin.

The autoradiographic results corroborate previous X-ray microanalytical data which demonstrated high levels of transition metals in the condensed chromatin of a variety of freshwater and marine dinoflagellates. These transition metals have been detected in chromatin within unfixed, air-dried cryosections (KEARNS and SIGEE 1980), dispersed washed cryosections (KEARNS and SIGEE 1980), and chemically fixed, embedded cells — where they are present as insoluble (bound) constituents. In chemically processed cells, transition metals were not detected in the cell cytoplasm (KEARNS and SIGEE 1979). Their presence in the nucleus was largely confined to the condensed chromatin, though small X-ray emission peaks were occasionally present in the nucleoplasm.

The present study was carried out to gain further information on the presence of transition metals in dinoflagellate chromatin, using a completely new (autoradiographic) approach. Within the transition metals, the radio-isotope of nickel, $^{63}$Ni, was chosen as being particularly suitable for autoradiography (see discussion). The experimental material, Glenodinium foliaceum (Stein), has been previously studied in terms of fine structure (DODGE 1971) and X-ray microanalysis (SIGEE and KEARNS 1981a), and is of particular interest in having two distinct types of nucleus in each vegetative cell.

Keywords: Autoradiography; Chromatin; Chromosome; Dinoflagellate; Nickel; Transition metal.

1. Introduction

Recent studies using X-ray microanalysis have demonstrated (KEARNS and SIGEE 1979) and quantified (KEARNS and SIGEE 1980, SIGEE and KEARNS 1981a, 1981b, 1981c) high levels of the transition metals Fe, Ni, Cu and Zn in the condensed chromatin of a variety of freshwater and marine dinoflagellates. These transition metals have been detected in chromatin within unfixed, air-dried cryosections (KEARNS and SIGEE 1980), dispersed washed cryosections (KEARNS and SIGEE 1980), and chemically fixed, embedded cells — where they are present as insoluble (bound) constituents. In chemically processed cells, transition metals were not detected in the cell cytoplasm (KEARNS and SIGEE 1979). Their presence in the nucleus was largely confined to the condensed chromatin, though small X-ray emission peaks were occasionally present in the nucleoplasm.

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2. Materials and Methods

2.1. Culture of Material

Glenodinium foliaceum (Stein), obtained from the Cambridge Culture Centre (ref. 1116/3), was grown in AE 50 liquid culture medium at 21°C, under a 12 hours light/12 hours dark regime. Asynchronous cultures were labelled with $^{63}$Ni during the log growth phase. The culture pH at the time of labelling was 8.3.
2.2. Labelling and Cell Preparation

50 ml of $^{63}$Ni chloride (Radiochemical Centre, Amersham) in 0.1 M HCl was adjusted to pH 8.3 by addition of 0.5 M Tris buffer, and was added to 50 ml of Glenodinium culture, giving a final concentration of 10 $\mu$Ci/ml (specific activity 10 mCi/mg Ni). Cells were labelled for periods of 2 hours, 4 hours (continuous light) and 24 hours (light/dark), collected by gentle centrifugation, and resuspended in one of the following fixatives for 2 hours at 20°C.

- (a) 2.5% glutaraldehyde (TAAB) in 0.1 M sodium cacodylate buffer, pH 7.2.
- (b) 2% paraformaldehyde, dissolved in 0.1 M sodium cacodylate buffer, pH 7.2, by heating to 70°C (4 hours $^{63}$Ni treatment only).
- (c) Acetic-alcohol (3 parts ethanol: 1 part acetic acid). 4 hours $^{63}$Ni treatment only.

After aldehyde fixation, cells were washed three times in cacodylate buffer, and were then dehydrated in an ethanol series. Cells fixed in acetic alcohol were transferred directly to pure ethanol, which was replaced three times. Cells were then embedded in SPURR (1969) resin, and sections cut at thicknesses of 6 $\mu$m and 10-15 nm for light and electron microscopy.

2.3. Autoradiography

For light microscope autoradiography 6 $\mu$m sections were coated with Ilford G 5 emulsion using a dipping technique, left for 14-21 days at 4°C, and processed using Kodak D 19 as developer. For electron microscope autoradiography, ultrathin sections were coated with a monolayer of Ilford L 4 emulsion using a wire loop, and left for a period of 3-4 months before processing in Kodak D 19 developer.

3. Results

3.1. Light Microscopy

Under the light microscope, the presence of two distinct nuclei in each cell was readily visible, irrespective of the type of fixation. The typical dinoflagellate (dinocaryotic) nucleus occurred as a single spherical structure, containing permanently condensed chromat (chromosomes); while the atypical (supernumerary) nucleus was observed as discrete lobes of uniform density (Figs 1a, 2a, and 3a). The major differences in fixation appearance occurred with the acetic-alcohol-fixed cells, which had a low overall density under phase-contrast, poor cytoplasmic preservation, and frequently had an irregular shape (Fig. 3a).

Examination of the light microscope autoradiographs revealed that all cells were active in the uptake of radioactive nickel — irrespective of fixation or duration of labelling. The heavily labelled cells typically showed a scattering of silver grains over the whole cell, with no clear localisation to any major cell component such as nuclei (Figs. 1, 2, and 3). Silver grains also occurred scattered around the cells as a halo.

The type of fixation did not appear to influence either the distribution (Figs. 1, 2, and 3) or the total number of silver grains over the cells. Mean grain counts over cells exposed to $^{63}$Ni for 4 hours, and then fixed in different ways (Table 1) did not show any significant differences between treatments.

The effect of increasing the duration of $^{63}$Ni labelling from 2-24 hours was investigated with glutaraldehyde-fixed cells (Table 1). Exposure of cells to $^{63}$Ni for a period of 2 hours lead to active incorporation of label. Increase in duration of labelling to 4 hours and 24 hours resulted in a significant but non-linear increase in the cell content of bound-$^{63}$Ni.

Table 1. Cell grain counts (light microscopy). Grain counts were made of the total number of silver grains lying directly over cell sections containing clear dinocaryotic and supernumerary nuclei. Each mean value is derived from a total of 20 cells, with background (estimated at 1 grain per cell) subtracted

<table>
<thead>
<tr>
<th>Fixation</th>
<th>Duration in $^{63}$Ni</th>
<th>Mean count per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde</td>
<td>2 hours</td>
<td>87.2 ± 12.8*</td>
</tr>
<tr>
<td></td>
<td>4 hours</td>
<td>119.5 ± 14.7</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>204.2 ± 12.8</td>
</tr>
<tr>
<td>Paraformaldehyde</td>
<td>4 hours</td>
<td>132.8 ± 13.1</td>
</tr>
<tr>
<td>Acetic alcohol</td>
<td>4 hours</td>
<td>119.5 ± 11.4</td>
</tr>
</tbody>
</table>

* 95% confidence limits.

3.2. Electron Microscopy

Electron microscope autoradiography was carried out on cells labelled for 2 hours with $^{63}$Ni, and fixed in glutaraldehyde or glutaraldehyde and osmium tetroxide. The use of relatively thick (10-15 nm) sections increased silver grain yield, but resulted in a reduced definition of cell membranes — including the nuclear envelope (Figs. 4, 6, and 7).

The distribution of silver grains differed from the light microscope preparations in showing a marked localisation to the dinocaryotic nucleus. Whereas a high frequency of grains consistently occurred over dinocaryotic nuclei (containing distinct profiles of chromosomes — Figs. 4–7), silver grains were only rarely seen over the supernumerary nucleus (containing a homogeneous reticulate chromatin — Fig. 8).

Grain counts over ten separate cells, taken from the same autoradiographic preparation, and selected simply for the presence of both dinocaryotic and supernumerary nuclear profiles in the same section, are