

Lectin-like components in the macronuclear replication bands of *Euplotes eurystomus*

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Upon incubation with fluoresceinylated neoglycoproteins, isolated macronuclei from the ciliated protozoan *Euplotes eurystomus* display different labelling patterns depending on the nature of the sugar bound to the neoglycoproteins. Specific sugar-binding components (*i.e.*, lectin-like molecules) are associated with presumed nucleoli and with the macronuclear replication bands. This is the first demonstration that DNA synthesis and sugar-binding components are co-localized in an eukaryotic cell.

nuclear lectin — neoglycoprotein — DNA synthesis — nucleolus — fluorescence microscopy

INTRODUCTION

Previous studies have demonstrated the actual existence of sugar-binding sites related to lectin-like proteins in mammalian cell nuclei [16, 17]. These nuclear lectins have been shown to be preferentially localized in nuclear and nucleolar areas known to be the sites of transcriptional and post-transcriptional events in reptilian and mammalian cells [4, 5, 7] but no sugar-binding sites were detected in the condensed chromatin [6]. However, the latter result does not rule out the existence of lectin activity during DNA synthesis. Since no specific structure allows localization of the site of DNA synthesis in higher eukaryotes, we used hypotrichous ciliated protozoa, the macronucleus of which have 2 unique features of interest with regard to eukaryotic chromatin structure and function [8, 14]: 1) short (average, 2 kbp) linear, highly endoreplicated DNA molecules with single coding regions; 2) microscopic migrating organelles, the replication bands (RBs) which are the principal sites of DNA synthesis. Extensive ultrastructural, cytochemical and immunochemical studies of RBs [1–3, 12, 13] have demonstrated the distinctive characteristics of RBs in comparison to adjacent non-replicating chromatin. Furthermore, *in vitro* deoxynucleotide incorporation into RBs has been demonstrated [14]. In the present paper, using fluoresceinylated neoglycoproteins [9, 10] that have been shown to be suitable to visualize

and quantitate nuclear lectins of mammalian cells [17], we show that some nuclear sugar-binding components possess spatial and temporal coincidence with sites of DNA and chromatin replication.

MATERIALS AND METHODS

Neoglycoproteins, prepared as described earlier [10] contained 25 ± 3 sugar units and were fluoresceinylated and characterized according to Roche *et al.* [15]. The fluoresceinylated neoglycoprotein used contained the following sugars: α -D-mannose (F-Man-BSA); α -D-mannose-6-phosphate (F-Man-6-P-BSA); α -D-glucose (F-Glc-BSA); α -L-fucose (F-Fuc-BSA); α -L-rhamnose (F-Rha-BSA) or *N*-acetyl- β -D-glucosamine (F-GlcNAc-BSA). Fluoresceinylated wheat germ agglutinin (F-WGA) and gorse lectin (F-UEA-1) as well as chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Euplotes eurystomus cultivation has been described previously [1]. Lysis of rapidly growing *Euplotes* and incubation with fluoresceinylated proteins were performed at room temperature as follows: to a small glass Petri dish was added in sequence: 1.0 ml concentrated cells; 12.5 μ l Metofane (methoxyflurane); 200 μ l 6 \times lysis buffer (*i.e.*, 1 \times lysis buffer consisting of 0.5% NP-40, 10 mM Pipes, 1 mM spermidine phosphate, 5 mM MgCl₂, pH 6.75). The dish was oscillated frequently for about 10 min, followed by several passages through a Pasteur pipette to complete release of macronuclei. Fluoresceinylated proteins dissolved at a stock concentration of 1.25 mg/ml in 1 \times lysis buffer were added to the lysed cells to yield a final concentration of 125 μ g/ml (unless otherwise stated). After a 30-min incubation at room temperature, the nuclei were cytospun onto polylysine-coated slides (3 min, 550 rpm), plunged for 5 min into PBS 3.7% in formaldehyde, rinsed 3 times with PBS, and

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mounted in 90% glycerol/PBS/DABCO [14]. Slides were observed on an Olympus BH microscope and photographed on Tri-X film.

RESULTS

A number of neoglycoproteins revealed very strong macronuclear staining (Fig. 1 and Table I). F-GlcNAc-BSA and F-Man-6-P-BSA gave particularly strong staining of replication bands (RBs), (Figs. 1 A, B, C, F). The RBs bound much less F-Rha-BSA or F-Fuc-BSA than the other neoglycoproteins (Table I). F-BSA used as a control did not bind to the macronuclei (Fig. 1 I). Positive controls were obtained by using F-WGA which did not label the RBs but stained other areas within the macronucleus (Figs. 1 K, L). In contrast, F-UEA-1 gives a dull staining of the whole macronucleus (not shown). Outside RBs, the labelling pattern was either punctate or lace-like, depending on the fluorescent marker used (Table I). A punctate pattern predominated with F-Glc-BSA and F-WGA (Figs. 1 G, L) while a lace-like pattern predominated with F-GlcNAc-BSA (Figs. 1 A, B) and F-Man-6-P-BSA (Figs. 1 D, E, F). The punctate pattern is believed to be correlated with the numerous macronuclear nucleoli [1, 13]; so, F-Glc-BSA could label the nucleoli of *Euplotes eurystomus* as well as those of mammalian cells [17]. The lace-like pattern is interpreted as corresponding to the spaces between condensed chromatin granules and nucleoli [13].

The labelling observed is sugar-specific for the following reasons: i) it is not due to a non-specific entrapment of the fluoresceinylated markers because the staining pattern is dependent on the nature of the sugar linked to the protein carrier, and because there was no difference of staining strength or staining pattern when the slides were fixed with formaldehyde before or after PBS washing; ii) although most incubations were performed with markers at a final concentration of 125 $\mu\text{g/ml}$, more dilute

marker solutions were tested. The pattern of staining never appeared to change; only the staining intensity decreased correlatively with the decrease of marker concentration. Strong reactions were still obtained at the following concentration: 0.2 μM (*i.e.*, 15 $\mu\text{g/ml}$) of F-GlcNAc-BSA or F-Man-6-P-BSA; 0.4 μM of F-Rha-BSA; 0.8 μM of F-Glc-BSA. The advantage of decreasing the concentration of the fluoresceinylated markers is to decrease the fluorescence glare. iii) The replication bands were strongly stained by F-GlcNAc-BSA, but not at all by sugar-free F-BSA. Furthermore, F-WGA, which gives a strong punctate pattern throughout the macronucleus, does not label the replication bands at all.

The present data show that sugar-binding components which have been observed in the nucleus of reptilian [7] and mammalian cells [17] are also present in a protozoan. In mammalian cells, the binding has been shown to be modulated in relation to the physiological state of the cells [11, 17]. Using labeled neoglycoproteins, these sugar-binding sites were localized in areas known to be related to transcriptional and post-transcriptional activities, specially in the nucleoli [4–6, 17]. The present paper shows that in *Euplotes eurystomus*, sugar-binding components are not only associated with presumed nucleoli or with areas between condensed granules and nucleoli but also with replication bands. Knowing that replication bands are both easily identifiable as microscopic organelles (see Fig. 1 J) and are the principal sites of DNA synthesis [12–14], the present data clearly demonstrate that sugar-binding components are active at the site of DNA replication. The significance of this precisely located lectin activity is not yet known, but according to the dynamic properties of the replication bands, the associated lectins might be involved in maintaining the integrity of RBs as they migrate through the macronucleus and/or in activating (or assisting) the directional propagation of RBs. Identification of the sugar-binding components of RBs can now be initiated with enriched RB fractions [2].

In conclusion, the presence of lectin-like proteins in the macronucleus of a protozoan as well as in the nucleus of mammalian and reptilian cells suggests that they are ubiquitous nuclear proteins. These lectin-like proteins could be involved not only in transcription and/or post-transcription processes as shown in mammalian cells, but also in DNA replication, at least in protozoans such as *Euplotes*.

TABLE I. — Binding of fluoresceinylated markers to *Euplotes* macronuclei.

Markers	RB ^a	Mac pattern ^b
F-GlcNAc-BSA	+++	+ (1)
F-Man-6-P-BSA	+++	+ (1 and p)
F-Man-BSA	++	+ (p and 1)
F-Glc-BSA	++	+ (p and 1)
F-Rha-BSA	+	+ (1)
F-Fuc-BSA	±	± (1)
F-BSA	—	—
F-WGA	—	+++ (p)
F-UEA-1	±	± (1)

^a: RB: replication band.

^b: Pattern of the macronucleus labeling: Mac; macronucleus; 1, lace-like; p, punctate.

+++ : very intense labeling; ++ : intense labeling; + : modest labeling; ± : faint labeling; — : no labeling.

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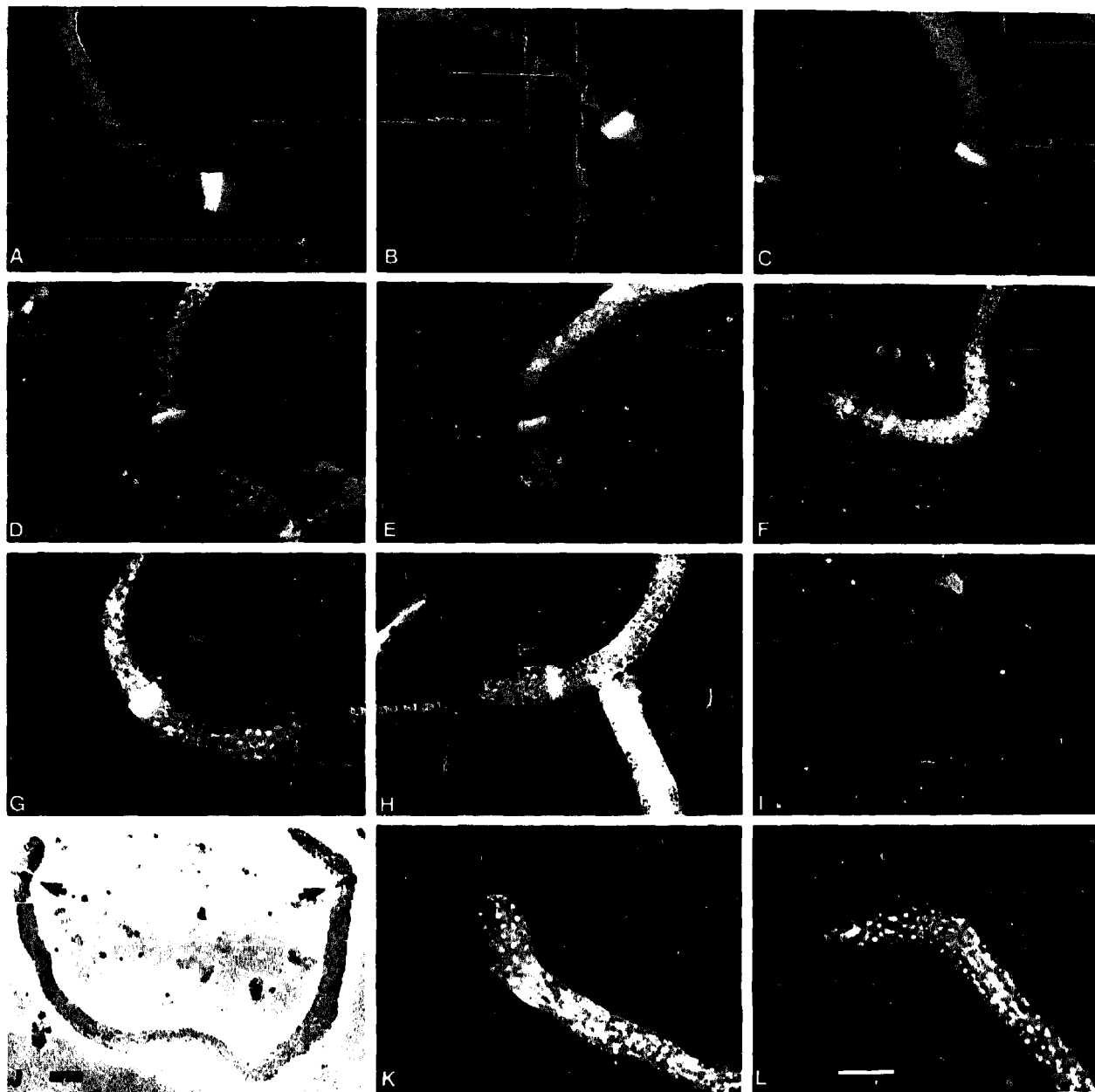


FIGURE 1. — Binding pattern of the fluoresceinylated markers within isolated *Euplotes* macronuclei. A–C, F-GlcNAc-BSA; D–F, F-Man-6-P-BSA; G, F-Glc-BSA; H, F-Rha-BSA; I, F-BSA (control); J–L, F-WGA. J is a phase micrograph of a single macronucleus exhibiting 2 RBs (arrows); K and L are the fluorescence micrographs of the 2 tips of the macronucleus shown in J. All fluorescence micrographs are presented at the same magnification; bar = 10 μ m.

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