Cytochemical and immunocytochemical study of coiled bodies in different cultured cell lines

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Abstract. We analyzed by different cytochemical and immunocytochemical approaches the biochemical composition of coiled bodies in three different cultured cell lines. Coiled bodies are stained by the AgNOR staining method and by the EDTA regressive staining method preferential for ribonucleoprotein (RNP). Using the in situ polyadenylate nucleotidyl transferase-immunogold technique or anti-RNA antibodies, we decisively demonstrated the presence of appreciable amounts of RNA in coiled bodies. Neither the in situ terminal deoxynucleotidyl transferase-immunogold technique nor anti-DNA antibodies revealed any DNA in coiled bodies. Coiled bodies thus appear as distinct regions of cell nuclei involved in some steps of RNA metabolism but not directly in RNA synthesis. Their relationships with the dense fibrillar component of the nucleolus and with interchromatin granule clusters are discussed.

Introduction

Coiled bodies (CBs) are morphologically well-defined structures of most cells (Ramon y Cajal 1903; Hardin et al. 1969; Monneron and Bernhard 1969; Lafarga et al. 1983; Lafarga and Hervas 1983; Brasch and Ochs 1992; Lamond and Carmo-Fonseca 1993) where they can undergo regulated cycles of assembly and disassembly during interphase and mitosis (Brasch and Ochs 1992; Andrade et al. 1993; Carmo-Fonseca et al. 1993). They are non-capsular, round to oval structures with a diameter of 0.3–1 μm, and consist of meandering threads loosely arranged in a coiled fashion.

Although their functional significance is as yet unclear, CBs have been implicated in extranucleolar RNA metabolism (Raska et al. 1991; Carmo-Fonseca et al. 1992; Andrade et al. 1993; Carmo-Fonseca et al. 1993; Lamond and Carmo-Fonseca 1993). They are thought to have a role in pre- and/or postsplicing activities such as preassembly of small nuclear ribonucleoproteins (snRNPs), degradation of introns, and snRNP recycling from postsplicing complexes (Carmo-Fonseca et al. 1993; Lamond and Carmo-Fonseca 1993).

Cytochemical studies have clearly revealed that CBs are proteinaceous in nature (Recher et al. 1972; Moreno Diaz de la Espina et al. 1982; Williams et al. 1985; Zareba-Kowalska 1989). Several proteins have been located in these elements by immunocytochemistry (Fakan et al. 1984; Andrade et al. 1991; Raska et al. 1991; Zamore and Green 1991; Carmo-Fonseca et al. 1993). In particular, Sm antigen has been demonstrated in CBs (Fakan et al. 1984).

On the other hand, although recent in situ hybridization experiments have clearly shown the presence of snRNPs in CBs, it is pertinent to note that cytochemistry has never unambiguously demonstrated the presence of RNA in CBs. Their high contrast with Bernhard’s (1969) staining method cannot be considered unquestionable evidence of their RNP nature (Monneron and Bernhard 1969; Moreno Diaz de la Espina et al. 1982; Lafarga et al. 1983; Lafarga and Hervas 1983; Zareba-Kowalska 1989) since this technique is preferential but not specific for RNP. Furthermore, no high-resolution autoradiographic study has revealed any presence of RNA in CBs (Moreno Diaz de la Espina et al. 1982).

Likewise, the presence of DNA in CBs is uncertain. On the basis of a few cytochemical studies (Moreno Diaz de la Espina et al. 1982; Zareba-Kowalska 1989; Raska et al. 1991), CBs were regarded as nuclear structures devoid of DNA. Using DNase-gold complexes, however, Williams et al. (1985), observed labeling over CBs in the nuclei of Pisum sativum root tip cells.

In the present study, CBs were investigated by means of ultrastructural cytochemistry in three different cell lines. To ascertain whether RNA, DNA, or both are present within the CBs of cell nuclei, we applied various cytochemical and immunocytochemical approaches for precisely identifying RNA and DNA at the ultrastructural level. Our results demonstrate the presence of considerable amounts of RNA but no DNA in the CBs.
Materials and methods

Biological materials

Ehrlich ascites tumor cells (tetraploid line ELT), collected from C57Bl mice bearing the tumor, were cultured as previously described (Lepoint and Bassleer 1973). HeLa and Hep-2 cells were grown in Eagle’s minimum essential medium (Gibco-BRL, Life Technologies, Gent, Belgium) supplemented with 10% fetal calf serum and 100 U/ml penicillin.

Fixation and embedding

Cultures of the cell lines were scraped off the dishes and centrifuged to form pellets. Small fragments of various pellets were fixed for 15–60 min at 4°C in 1%–1.6% glutaraldehyde or 4% formaldehyde, 0.1% glutaraldehyde or 4% formaldehyde or 2.5% glutaraldehyde, 2% osmium tetroxide in 0.1 M Sorenson’s buffer, pH 7.4. They were dehydrated through graded ethanol or acetone solutions, and embedded in Epon or in Lowicryl K4M as in Roth et al. (1981). Some 60 min 1.6% glutaraldehyde-fixed fragments were acetylated according to Wassef et al. (1979). Other 15 min 1.6% glutaraldehyde-fixed fragments were rinsed in Sorenson’s buffer, fixed for 5 min at 4°C in Carnoy’s solution (1: 3 acetic acid:ethanol), rehydrated in graded ethanol (5 min each in 100%, 75%, 50% and 30%), incubated for 10 min at 60°C in a freshly prepared solution of 2% gelatin in 1% formic acid and 50% 5% thiosulfate solution, and finally rinsed in distilled water before being acetylated as previously described (Ploton et al. 1984).

Ultrathin sections of the various blocks were collected in platinum rings (4 mm diameter) formed by a platinum wire (0.1 mm diameter, SA Johnson Matthey NV, Brussels, Belgium) and stored in distilled water until used, or mounted on collodion-coated nickel grids. Finally, ultrathin sections were stained with uranyl acetate and lead citrate before examination in a Jeol CX100 electron microscope at 60 kV.

Detection of RNA

EDTA regressive staining. Some grid-mounted sections of acetylated cells were preferentially stained for RNP by Bernhardt’s (1969) method.

In situ polyadenylate nucleotidyl transferase (PnT)-immunogold procedure according to Thiry (1993a). Ultrathin sections of Epon-embedded cells were incubated for 5 min at 37°C on the surface of the following medium: 50 mM Tris-HCl, 10 mM β-mercaptoethanol, 10 mM MgCl₂, 2.5 mM MnCl₂, 0.25 M NaCl, 1 mg/ml bovine serum albumin (BSA), pH 7.9, 25 U/ml Escherichia coli PnT (Gibco BRL, Mereleke, Belgium), and 0.2 mM biotinyl-17-ATP (Sigma, St Louis, Mo.). Grids were incubated by floating them, cell sections down, on the same medium. After five rinses in bidistilled water, the different sections or grids were incubated for 30 min in PBSB (34 mM NaCl, 0.7 mM KCl, 20 mM NaHPO₄, 10 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 1% BSA, pH 7.2) containing normal rabbit serum (NRS) diluted 1/10. The next step of the treatment was a 60 min incubation at room temperature with goat anti-biotin antibodies (Biosys SA, Compiègne, France) diluted 1/500 in PBSB containing NRS diluted 1/50. After four rinses in PBSB, one cm² of rabbit anti-goat IgG showed a striking preference for synthetic dsRNA molecules (Daigne et al. 1998). For labeling, ultrathin sections of Lowicryl-embedded cells were incubated for 25 min in PBSB, pH 7.2 containing normal goat serum (NGS) and NRS, each diluted 1/30, and then for 3 h at room temperature in both RNA-specific antibodies diluted 1/10 in PBSB containing NGS and NRS, each diluted 1/50. After five rinses in PBSB, the sections were incubated for 30 min with goat anti-mouse IgG3 (heavy chain specific; Sigma, St Louis, Mo.) diluted 1/100 in PBSB containing NGS and NRS, each diluted 1/50. After four rinses in PBSB, pH 7.2 plus one in PBSB, pH 8.2, sections were transferred to an incubation medium containing rabbit anti-goat IgG coupled to colloidal gold 5 nm in diameter (Janssen Life Sciences) diluted 1/50 in PBSB, pH 8.2. Incubation was for 60 min at room temperature. Samples were then rinsed with PBSB followed by distilled water. This labeling was systematically applied to both faces of ultrathin sections.

Several kinds of control experiments were carried out. When the primary or secondary antibody or both were omitted, the ultrathin sections were devoid of label. When the grids were incubated with antibody-free particles, no labeling occurred. Finally, no label was detected when Lowicryl sections were preincubated at 56°C for 120 min with 1 mg/ml RNase A (Boehringer) in 10 mM Tris-HCl, pH 7.4 containing 15 mM NaCl.

Detection of DNA

Terminal deoxynucleotidyl transferase (TdT)-immunogold method according to Thiry (1992a). Ultrathin sections were incubated for 10 min at 37°C on the surface of the following medium: 20 μM 5-bromo-2-deoxyuridine (BUDR) triphosphate (Sigma, St Louis, USA), 100 mM sodium cacodylate, pH 7.2, 2 mM CoCl₂, 10 mM β-mercaptoethanol, 50 μg/ml BSA and 125 U/ml calf thymus TdT (Boehringer Mannheim, Germany). Sections were then incubated for 10 min at 37°C in the same medium supplemented with 4 μM considering that only antigenic determinants present at the surface of the sections are accessible to immunoglobulin-gold complexes (Bendayan and Stephens 1984), the in situ PnT immunogold procedure was systematically performed on each of the two faces of ultrathin sections. Once labeled on one side, the sections were mounted on collodion-coated nickel grids and in situ PnT-immunogold procedure was applied to the second face.

The specificity of the PnT reaction was tested in several ways. When PnT or biotinylated ATP was omitted from the PnT medium, the ultrathin sections were devoid of label. When either of the two ions was omitted from the PnT medium, labeling was strongly reduced.

The specificity of RNA labeling was also tested in several ways. When acetylated cell sections were preincubated at 36°C for 120 min with 1 mg/ml of pyrimidine-specific RNase (RNase A, Boehringer) in 10 mM Tris-HCl, pH 7.4 containing 15 mM NaCl, labeling was strongly reduced. The result was identical when these sections were preincubated at 37°C for 60 min with 500 U/ml of a purine-specific RNase (RNase T2, Gibco BRL) in 50 mM sodium acetate buffer, pH 4.5 containing 2 mM EDTA. No label was detected on acetylated cell sections pretreated with RNase T2, followed by RNase A. Preincubation at 37°C for 120 min with 1 mg/ml DNase I (Sigma, type DN-Ep) in PBS (0.14 M NaCl, 6 mM Na₂HPO₄, 4 mM KH₂PO₄, pH 6.8) containing 7 mM MgCl₂ did not prevent labeling. Finally, when RNase T2 incubation was carried out after the PnT reaction, no labeling occurred.

The immuno labeling specificity was also tested. When the primary antibody was omitted, no labeling occurred. Gold lacking the antibody tag did not bind to the sections.

Immunocytochemical technique for RNA. Two monoclonal mouse anti-RNA autoantibodies (D444, BWR5) were used (Eilat et al. 1988; Eilat and Fischel 1991). The isotype was IgG3.K. The D444 antibody was specific to a G+C-rich polyribonucleotide sequence, while the BWR5 IgG showed a striking preference for synthetic dsRNA molecules (Eilat et al. 1988).

For labeling, ultrathin sections of Lowicryl-embedded cells were incubated for 25 min in PBSB, pH 7.2 containing normal goat serum (NGS) and NRS, each diluted 1/30, and then for 3 h at room temperature in both RNA-specific antibodies diluted 1/10 in PBSB containing NGS and NRS, each diluted 1/50. After five rinses in PBSB, the sections were incubated for 30 min with goat anti-mouse IgG3 (heavy chain specific; Sigma, St Louis, Mo.) diluted 1/100 in PBSB containing NGS and NRS, each diluted 1/50. After four rinses in PBSB, pH 7.2 plus one in PBSB, pH 8.2, sections were transferred to an incubation medium containing rabbit anti-goat IgG coupled to colloidal gold 5 nm in diameter (Janssen Life Sciences) diluted 1/50 in PBSB, pH 8.2. Incubation was for 60 min at room temperature. Samples were then rinsed with PBSB followed by distilled water. This labeling was systematically applied to both faces of ultrathin sections.

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