Micromorphology of cytoplasmic nucleoprotein complexes harboring an extrachromosomal DNA closely related to avian myeloblastosis virus core-bound DNA

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Abstract **Nucleoprotein (NP) complexes constituting the three basic components (A, B, C) of the postmicrosomal sediment (POMS) of chicken leukemic myeloblasts (CHLMs) which contain extrachromosomal DNA closely related to avian myeloblastosis virus DNA were analyzed electron microscopically. It was shown that these NP complexes resemble micromorphologically, depending on the origin of their POMS components, NP structures involved in three successive stages of early DNA synthesis. Nucleic acids harbored in these NP complexes exhibited micromorphological features typical for replicative structures. It was confirmed electron microscopically that the extrachromosomal DNA of CHLMs replicative in nature and of three length classes is organized into special NP complexes, each of which, as demonstrated, represents a unique reaction machinery of early DNA synthesis.**

Key words: Avian myeloblastosis virus DNA; Replicative structure; Nucleoprotein complex; Micromorphology

1. Introduction

Avian myeloblastosis virus (AMV), like other retroviruses [1–4], contains a 'small' host DNA [5]. Only recently, it has been recognized that such virus core-bound [6,7] AMV DNA actually represents minute early replicative structures [8–10] associated with primase (Pr) and Pr-α DNA polymerase (pol) activities [11]. Accordingly, this implies the possible participation of this DNA in association with special cell proteins in replication and/or integration activities of the retrovirus core reaction machinery [12]. To elucidate this question, we need to know more about, among others, the properties of nucleoprotein (NP) complexes into which this DNA is organized prior to its association with the virus core components. To study this problem, we used the material of the postmicrosomal sediment (POMS) of chicken leukemic myeloblasts (CHLMs) which contains DNA identical to AMV DNA in physico-chemical and hybridization properties [13], in specific radioactivity [9], and micromorphologically [10]. Electron microscopically it also resembles the cytoplasmic small polydisperse linear and circular DNA of growing animal cells [14,15]. Experiments done in this direction have shown that the POMS material of CHLMs is formed by several populations of NP complexes constituting the three basic POMS components (A, B, C) differing from each other in sucrose density, sedimentation properties and in properties of radioactive labelling for DNA and RNA. The sedimentation and electrophoretic characteristics of the radioactively double-labelled nucleic acids (NAs) of the NP complexes representing the individual POMS components were shown to be reminiscent of the intermediates of early DNA synthesis, minimally, medium and maximally advanced in NP complexes of POMS components C, B and A, respectively [16]. In addition, these data indicated that NAs of POMS components B and C are responsible for representation of the major and minor portions, respectively, of molecules constituting AMV DNA [8,9]. These findings were complemented by data showing that the POMS NP complexes are associated with distinct NA synthesizing activities significant for initiating and more advanced stages of lagging DNA strand synthesis [17], as confirmed by analysis of the products of reactions accomplished in vitro by NP complexes of the individual POMS components [18]. Consequently, all these characteristics offer a unique opportunity to characterize micromorphologically the indicated replicative metabolic nature of the individual NP complexes descending from the individual POMS components as well as the NAs which these NP complexes harbor. Here, we present results obtained with this analysis.

2. Materials and methods

2.1. Separation of NP complexes, isolation of NAs

Starting material for separation of NP complexes in equilibrium sucrose density gradients [16] (see Fig. 1) was POMS material of CHLMs grown in tissue culture [1] and double-labelled for DNA and RNA as described [8]. The POMS material used was void of cell organelles including ribosomes and represented material of the cytoplasmic fraction that remains in suspension in the $138000\times g$ supernatant of the cell lysate [16] centrifuged for 1 h at 4°C and that turns into sediment (POMS) during a further 1 h centrifugation at $230000\times g$. Consequently, to maintain the POMS material in suspension, we separated it directly from the cell lysate $138000\times g$ supernatant by centrifugation of aliquots in isopycnic gradients (Fig. 1). Estimation of DNA, RNA and protein content of POMS material present in the selected gradient fractions was accomplished as described [16]. NAs of POMS material of selected gradient fractions were isolated after dialysis and SDS-proteinase K treatment [16] by phenol-chloroform extraction and ethanol precipitation.

2.2. Electron microscopy

Two different electron microscopy techniques for visualization of NP complexes and NAs were used to eliminate the influence of the visualization method on their structure and the length distribution profile: (a) a modified BAC spreading technique [19] and (b) the technique of activated carbon-coated grids [20]. Electron micrographs were recorded at a magnification of 10000 or 15000 in a JEOL JEM 1200EX microscope operating at 60 kV. Length measurements of the molecules were performed with an Olivetti PD3 digitizer using pBR322 and pUC18 DNAs as internal length standards.

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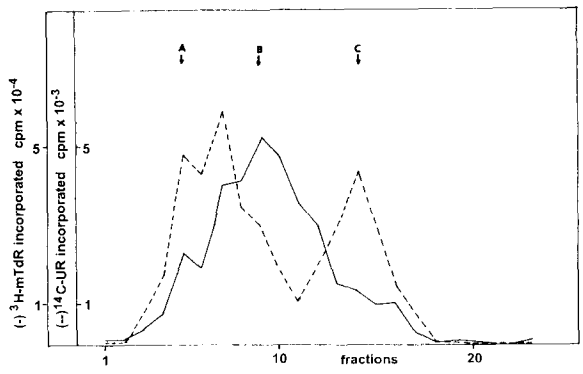

Fig. 1. Equilibrium density gradient centrifugation of POMS material (separated from the cell lysate 138 000×*g*) in linear 20–60% sucrose gradient in a buffer at 147 000×*g* for 21 h at 4°C. The individual fractions were used for ^{3}H and ^{14}C radioactivity assay and selected peak fractions (designated A, B and C) for estimation of DNA, RNA and protein content as well as for micromorphological analysis of NP complexes and NAs.

3. Results and discussion

The [^{3}H]mTdR and [^{14}C]UR double-labelled POMS material of CHLMs consists of several populations of NP complexes constituting the three basic POMS components (A, B, and C) which can be separated in sucrose density isopycnic gradients (Fig. 1). While the POMS components A and C are markedly labelled for RNA with the [^{14}C]UR radioactivity peak at densities of 1.219 and 1.108 g/cm^{3}, respectively, the POMS component B is maximally labelled for DNA with a [^{3}H]mTdR radioactivity peak at a density of 1.180 g/cm^{3}. NP complexes derived from the radioactivity peak fractions of the individual POMS components differed micromorphologically. NP complexes of POMS component A, rich in RNA in comparison to their content of DNA and with an almost equal NA/protein proportion (Table 1), represent electron microscopically typical compact structures (Fig. 2A). Most of these NP complexes reveal the presence of large electron-dense centers with a diameter in the range of 0.1–0.4 μm (on average 0.29–0.5 μm), suggesting their protein nature. The centers are surrounded by randomly oriented strands of heterogeneous length ranging from 0.25 μm to 1.30 μm (Fig. 2A, insert). Most of these strands representing NA molecules usually revealed the double-stranded nature but were not devoid of single-stranded gaps, as can be assumed from their appearance in different types of spreading. These NP complexes possess NA synthesizing activities which, using the endogenous template DNA, synthesize DNA of 250–280 bases in length, reminiscent of mature Okazaki fragments [16,18].

NP complexes of POMS component B, in contrast to those of POMS component A, rich in DNA and exhibiting a two-fold increase in their protein content (Table 1), reveal a different micromorphology. They differ markedly from those of POMS component A as well as C by pronounced unravelling of their structures reflecting their high replicating activities monitored already by their outstanding properties of labelling for DNA [16] (see Fig. 1). Electron-dense centers typical of NP complexes of both A and C POMS components were in this case rather diffuse (Fig. 2B). This may indicate an increased incidence of pre-initiation structures in which the origins are locally unwound [21]. Accordingly, the occurrence of single-stranded NAs associated with small protein structures in these NP complexes is distinctly increased (Fig. 2B). These NP complexes, as shown earlier, are equipped with two DNA pol activities, one represented by Pr-α DNA pol and the other by proliferating cell nuclear antigen insensitive δ DNA pol [17], which, mutually linked, lead to the synthesis of DNAs 140 bases in length reminiscent of Okazaki fragments [18].

NP complexes of POMS component C, again rich in RNA and exhibiting, in comparison to those of POMS component A, a 10-fold increase in protein content (Table 1), were micromorphologically similar to a certain degree to those of POMS component A. However, the NP complexes were significantly smaller in diameter and their electron-dense centers, sometimes diffuse in appearance (Fig. 2C, insert), were clearly larger (Fig. 2C). Such augmented structures of protein nature may indicate the presence of a set of proteins locally associated with sites of initiation of DNA replication [21]. NA strands surrounding these centers were only rarely observed (Fig. 2C).

In conclusion, the micromorphological characteristics of NP complexes of all three POMS components are strongly reminiscent of various stages of early DNA replication studied electron microscopically in prokaryotic systems [22].

Earlier, we characterized electron microscopically the NAs isolated from the total unfractionated POMS material [10]. Here, we present the micromorphological characterization of NAs isolated from NP complexes of the individual POMS components (Fig. 3). The NAs of NP complexes of POMS component A are represented mostly by linear double-stranded molecules, a minor portion of which is bent (Fig. 3A, arrow). However, single-stranded NA molecules were also detected (Fig. 3A, arrowhead). Some molecules of these NAs revealed an appearance typical of replicative structures [19] as shown in the insert (Fig. 3A). The length distribution of these NAs is apparent from the histogram (Fig. 4, fr. A) showing molecules with a length ranging from 0.02 to 0.17 μm with a mean length of 0.069 ± 0.025 μm corresponding to a molecular mass of 205 bp. In contrast, the NAs from NP complexes of POMS component B were substantially enriched in single strands (Fig. 3B) and a greater part of these NA molecules were bent. In this case typical replicative NA structures were detected more frequently (Fig. 3B, insert) in accordance with the highly replicative metabolic nature of NP complexes of POMS component B [16,18]. The length distribution of these NAs (Fig. 4, fr. B), ranging from 0.02 to 0.17 μm, showed a mean length of 0.055 ± 0.027 μm corresponding

Table 1
Composition and sucrose density of NP complexes from the peak fractions of the POMS components A, B and C from equilibrium density gradient (Fig. 1)

POMS component	Sucrose density g/cm^{3} (%)	DNA (μg)	RNA (μg)	Acid-soluble material (NTs) (μg)	Protein (μg)	NAs (μg)/protein (μg)
A	1.219 (49.5)	2.79 ± 0.21	16.2 ± 0.63	0.085 ± 0.002	16.21 ± 0.94	1.16
B	1.180 (42.0)	12.40 ± 0.56	9.08 ± 0.46	0.085 ± 0.002	45.50 ± 2.86	0.49
C	1.180 (26.5)	3.28 ± 0.69	7.40 ± 0.42	0.099 ± 0.003	84.76 ± 8.32	0.12

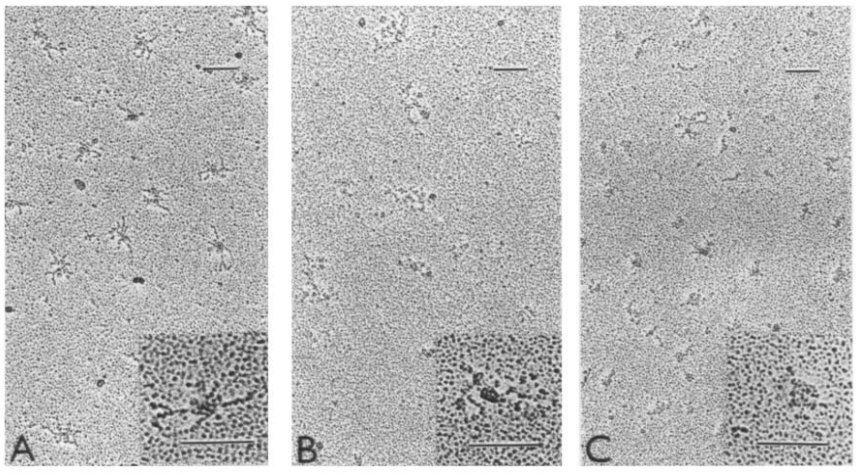

Fig. 2. Electron micrographs of NP complexes from the peak fractions of POMS components A, B and C from equilibrium density gradient. Bars represent 100 nm.

to a molecular mass of 160 bp. For comparison, the lengths of the most prominent DNA species synthesized by these NP complexes during the period of radioactive labelling of CHLMs [16] and of that synthesized in reaction in vitro [18] were found to be 150 bp and 140 bp (analyzed under denaturing conditions), respectively. The lengths of the majority of AMV DNA molecules, analyzed biochemically [9] and micromorphologically [10], were found to be 160 and 140 bp, respectively. Consequently, NAs of the POMS component B most active in replicative activities [16] are evident candidates for the major portion of AMV DNA molecules.

As the incidence of bent structures increases gradually from NAs of NP complexes of POMS component A through B to those of POMS component C, NA molecules bent up to circularization are typical of the NAs of POMS component C (Fig. 3C, arrow and insert). Similar circular NAs have also been found in AMV DNA [10], suggesting together with other data [16–18] that a minor portion of AMV DNA molecules [8,9] originates from the NAs of NP complexes of this POMS component. Besides the bent strands, also short linear strands can be recorded in these NAs (Fig. 3C, arrowhead). The presence of very short NAs, electron microscopically undetectable, is apparently responsible for a higher background of these NA preparations. These very short NAs, a few nucleotides long, may represent the reaction product of Pr and Pr-α DNA pol activities associated with NP complexes of POMS component C [17,18]. The length of NAs of NP complexes of POMS component C (Fig. 4, fr. C), ranging from 0.01 to 0.07 μm, exhibits a mean length of 0.035 ± 0.011 μm, corresponding to a molecular mass of 100 bp.

As regards the electron microscopical analysis of the NAs of POMS material, it does not allow, in general, detection in double-stranded structures of the possible occurrence of minute single-stranded gaps, the presence of which is indicated by the sensitivity of these structures to S1 nuclease (unpublished observation). Nevertheless, the presented micromorphological characteristics complement the biochemical data [16–18] obtained on this subject. Moreover, demonstrating micromorphologically the replicative metabolic nature of the NP complexes harboring the cytoplasmic extrachromosomal DNA of CHLMs, these characteristics show that each of these NP complexes represents, depending on the origin of its POMS components, a highly specialized reaction machinery of early DNA synthesis.

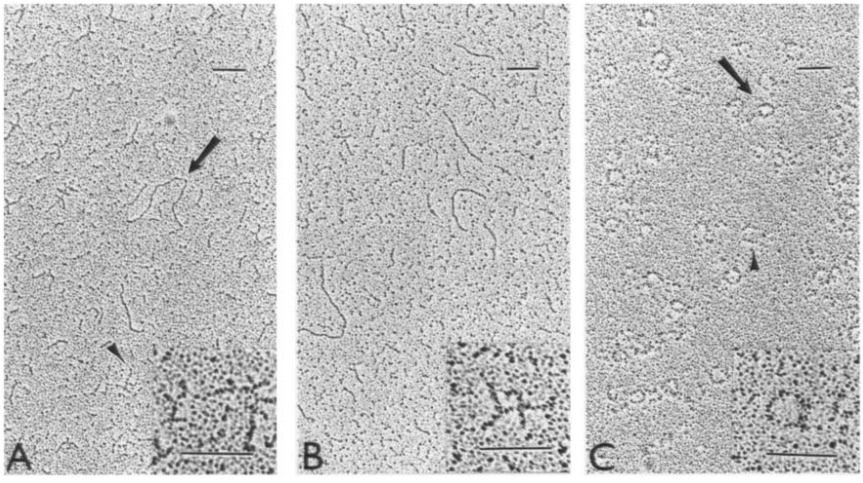

Fig. 3. Electron micrographs of NAs isolated from NP complexes of the peak fractions A, B and C. A: Minor portion of double-stranded NA molecules in POMS component A was bent (arrow) and some amount of single-stranded NAs was also detected (arrowhead); C: The presence of circular NAs is typical for POMS component C (arrow), but short linear strands can be also seen (arrowhead). Bars represent 100 nm.

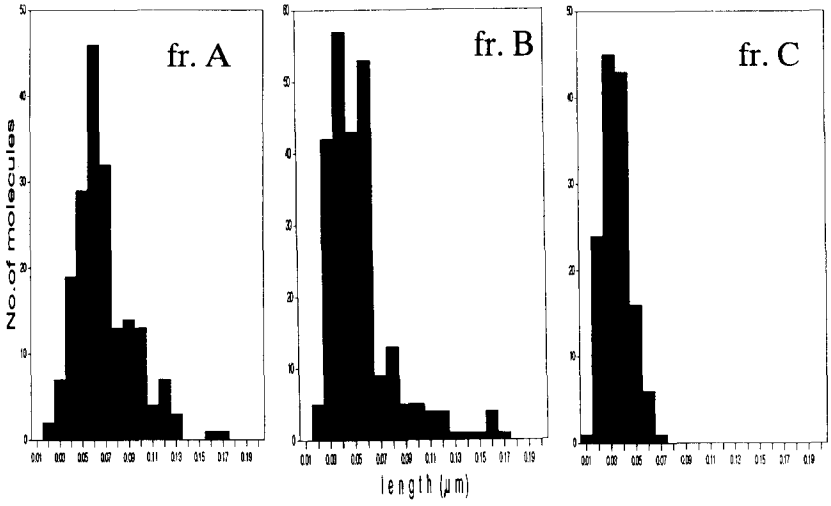

Fig. 4. Length distribution profile of NAs shown in Fig. 3.

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