Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 335-339

SUB-SET CHARACTERISTICS OF DNA SEQUENCES INVOLVED IN TIGHT DNA/ POLYPEPTIDE COMPLEXES AND THEIR HOMOLOGY TO NUCLEAR MATRIX DNA

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Received June 16, 1987

Polypeptides co-isolating with DNA induce the binding of a fraction of native DNA fragments to nitrocellulose filters. Southern analysis reveals a high intensity of self-hybridization of the DNA sequences retained on nitrocellulose filters. Consistently, the DNA fraction passing the filters shows only weak hybridization when probed with DNA retained on filters. This indicates that the DNA/ polypeptide complexes reside on a non-random sub-set of DNA sequences. Moreover, a high degree of homology was found between residual nuclear matrix DNA sequences and the DNA sequences retained on nitrocellulose filters. This indicates that the DNA sequences associated with tightly bound polypeptides originate from sites where the genome is salt-stably anchored in the nuclear matrix.

DNA isolated by various methods is still associated with DNA/ polypeptide complexes which can be radiolabelled either with ^{125}Iodine [1-4] or with ^{32}P during prolonged nick-translation (preceeding paper). The chemical stability of these complexes and their sensitivity to phosphodiesterases are consistent with phosphodiester bonds between internal DNA ends and O^4-hydroxyl groups of tyrosines in residual peptides [5].

With respect to the biological significance of this DNA/ polypeptide interaction it was of interest to investigate whether these complexes are randomly associated with DNA or whether they reside on a sub-set of DNA sequences which may be related to other DNA fractions, e.g those considered to be involved in attachment sites of DNA in high-salt insoluble nuclear structures. Since DNA fragments associated with the tightly bound polypeptides are specifically retained on nitrocellulose filters [6] this could be investigated by hybridization technology: If the residual peptides were associated randomly with all DNA sequences of the genome one should expect the same sequence complexity in unfractioned DNA and in the fractions retained on nitrocellulose filters. In contrast, if the residual peptides were bound to a sub-set of DNA sequences one should expect a lower sequence complexity in the DNA fraction retained on filters compared to unfractioned DNA.

0006-291X/87 $1.50

Methods

Isolation of DNA: DNA was isolated from Ehrlich ascites cells, nuclear matrix of Ehrlich ascites cells and nuclei pretreated with 2M salt and restriction endonuclease according to the method of Gross-Bellard et al. [7]. This method includes digestions with proteinase K in the presence of SDS, RNase treatment and phenol extractions.

Nuclear matrix of Ehrlich ascites cells was prepared by extraction of nuclei with DNase I, low salt and high salt (2 M NaCl) buffers as described previously [8,9].

Preparation of 'supernatant DNA' and 'pellet DNA' from halo-structures: Isolated nuclei of Ehrlich ascites cells were histone-depleted by treatment with a buffer containing 2 M NaCl as described by Keppel [10]. The pellet of the resulting halo-structures was washed twice in buffer containing 60 mM NaCl and 60 mM Tris-HCl, pH 7.4. This was followed by digestion with Alu I endonuclease in the same buffer supplemented with 5 mM magnesium chloride. The digestion was controlled by fluorescence microscopy in the presence of 1 μg per ml of ethidium bromide. After solubilization of the DNA outside of the residual nuclei the latter were collected by centrifugation. The DNA contained in the pellet fraction (27%) and that contained in the supernatant fraction (73%) was further purified according to Gross-Bellard et al. [7]. The purified DNA fractions were redigested with Alu I before gel electrophoresis and transfer to nitrocellulose membranes.

Fractionation of DNA by nitrocellulose filtration: DNA (20-30 OD_{260} units) was digested with Alu I endonuclease. The fragmented DNA was extracted with phenol and precipitated with ethanol. The DNA pellets were suspended in 3 ml of filtration buffer (0.5 M KCl, 5 mM EDTA, 10 mM Tris-HCl, pH 7.4). The mixtures were passed (by means of a disposable syringe) through nitrocellulose filters (BA85, Schleicher & Schuell, 2.5 cm) presoaked with filtration buffer and supported by disposable filter holders (FP 025/1, Schleicher & Schuell). The filters were washed by pressing through an additional volume (3 ml) of filtration buffer. DNA passing the filters was precipitated by ethanol, washed with 70% (v/v) ethanol and dried. The DNA retained on filters was eluted for alkaline agarose gel electrophoresis with 3x1 ml of 50 mM sodium hydroxide (37°C). The recovery of DNA bound to filters was in the order of 80%. When native DNA had to be recovered from filters for radiolabelling of the filter bound DNA by nick-translation the elution was performed with 3x1 ml of elution buffer (5 mM EDTA, 10 mM Tris-HCl, pH 7.4). In this case the recovery of DNA was in the order of 60%. DNA recovered from filters was concentrated by ethanol precipitation.

Southern hybridizations: Equal amounts of unfractioned DNA, DNA passing the filters, DNA eluted from filters by alkali, pellet DNA and supernatant DNA were submitted to alkaline agarose gel electrophoresis. Electrophoresis and transfer of DNA to nitrocellulose membranes (BA85; Schleicher & Schuell) were performed according to protocols suggested by Maniatis et al. [11]. DNA was radiolabelled to a specific radioactivity of $3x10^8$ cpm/μg by nick-translation with deoxycytidine 5'-[α-^{32}P]triphosphate (3000 Ci/mmole; N 5000 Nicktranslation Kit; Amersham, U.K.). Prehybridizations (4 hours) and hybridizations (18 hours) were performed in roller tubes at 68°C in buffers of the following composition: 0.4 M disodium hydrogen phosphate, pH 7, 3x Denhardt solution and 0.5% SDS. Following the hybridizations the blots were washed with 2xSSC, 0.5% SDS (twice for 30 min, 68°C), 0.1xSSC, 0.5% SDS (2 hours, 68°C) and 0.1xSSC, 0.5% SDS (30 min, 68°C). After drying the blots were exposed to Kodak X-Omat films and intensifier screens.

Results

Subset-characteristics of DNA sequences retained on nitrocellulose filters during filtration of Alu I digested DNA.

According to previously published data a fraction of 13% of native DNA is retained on

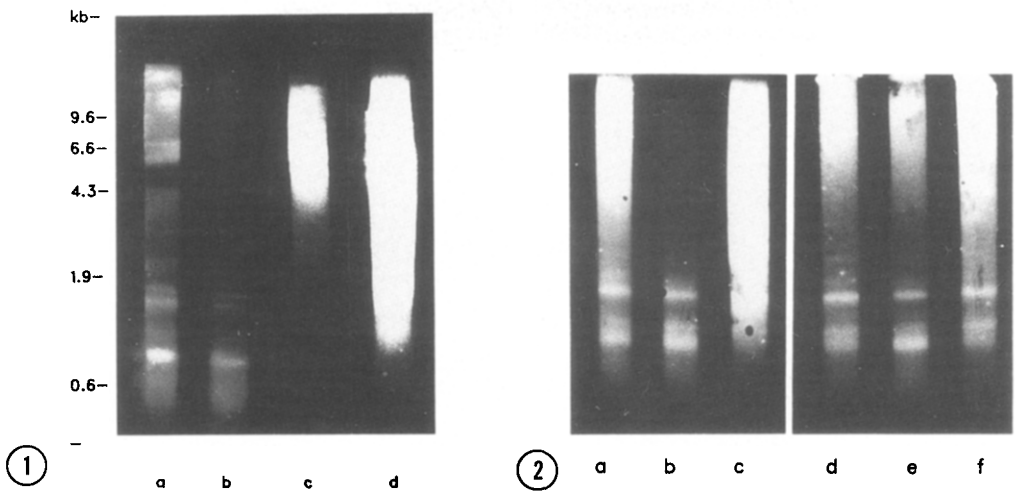

Figure 1. Southern hybridization of Alu I digested DNA from Ehrlich ascites cells with radiolabelled subset–DNA recovered from nitrocellulose filters. (a) Unfractioned DNA (0.1 μg); (b) DNA passing the filter (0.1 μg); (c) sub–set DNA recovered from filters (0.01 μg) and (d) (0.1 μg). Following electrophoresis on an alkaline agarose gel (1% w/v) the DNA was transferred to a nitrocellulose membrane which was probed with 2 μg of radiolabelled sub–set DNA recovered from filters.

Figure 2. Southern hybridization of Alu I digested DNA from Ehrlich ascites cells with radiolabelled residual nuclear matrix DNA from Ehrlich ascites cells. (a) Unfractioned DNA (1 μg); (b) DNA passing the filter (1 μg); (c) DNA recovered from filters (1 μg); (d) unfractioned DNA (1 μg); (e) supernatant DNA after Alu I digestion of halo–structures (1 μg); (f) 'pellet DNA' retained in the pellet after Alu I digestion of halo–structures (1 μg). Gel electrophoresis and Southern analysis were performed as described in the legend of Fig. 1.

nitrocellulose filters during filtration of Alu I digested bulk DNA due to its association with residual polypeptides [6]. Southern blots of unfractioned DNA, DNA retained on filters and DNA passing the filters were hybridized with DNA radiolabelled after its recovery from filters. It is shown in Fig. 1 that the hybrid formation between the radiolabelled DNA probe representing the complexity of the DNA sequences retained on filters is much higher with DNA recovered from filters than that with unfractioned DNA. Consistently, the hybrid formation of the radiolabelled DNA probe with the fraction of DNA passing the filter is weak.

Since the amount of DNA on the blot shown in Fig.1 is equivalent to the DNA of 10^4 – 10^5 genomes it follows that in most of the genomes of a population of identical cells the residual peptides inducing the binding of DNA fragments to nitrocellulose filters have to reside essentially on the same non–random sub–set of DNA sequences. If there would exist significant variations in these sub–sets between individual cells one would expect randomisation resulting in the same sequence complexity in the various fractions.

However, some cross–hybridization between the DNA sequences retained on filters and those passing the filters is observed (Fig. 1 b). This result has to be expected because of the following reasons: (1) The separation of the two DNA fractions cannot be

expected to be 100% complete. (2) Because of the wide distribution of some repetitive DNA sequences in the genomes [12] repetitive DNA sequences may occur accidentally on both, protein associated DNA fragments found in the filter DNA fraction and protein free fragments passing the filter. The relatively strong and distinct hybridization signals seen in Fig. 1 b seem to reflect such repetitive sequences. (3) As shown previously [6], actively replicating DNA sequences are enriched in the filter fraction. Consequently, in replicating cells, all DNA sequences of the genome have to belong transiently to the filter fraction. Since these experiments were performed with DNA from asynchronous cells the DNA retained on filters may comprise also some DNA sequences involved in non-constitutive, dynamic DNA/ peptide interactions depending on physiological processes of individual cells or of subpopulations of cells.

However, despite of such accidental or physiological causes it appears that the fraction of DNA sequences passing the filters but hybridizing with DNA recovered from filters is low. This indicates that the factors inducing the binding of DNA fragments to nitrocellulose filters reside on a sub-set of DNA sequences.

The binding of a sub-set of DNA sequences to nitrocellulose filters is not restricted to mouse DNA isolated from Ehrlich ascites cells. The low sequence complexity of the filter DNA fraction was found also for Alu I digested DNA from differentiated cells, e.g. hen oviduct cells and chicken erythrocytes (results not shown).

Hybrid formation between residual nuclear matrix DNA and the sub-set of DNA sequences retained on filters

Residual nuclear matrix DNA (about 1% of the genomic DNA) which is considered to comprise the attachment sites of the genome in the nuclear matrix [13] was used as a probe for typical DNA sequences proximally involved in such attachment sites. It was found that the DNA sequences in bulk DNA hybridizing with the nuclear matrix DNA sequences (Fig. 2 a) are almost quantitatively retained on nitrocellulose filters (Fig. 2 c). The distinct hybridization signals found with the DNA fraction passing the filter (Fig. 2 b) point to repetitive DNA sequences occuring in the nuclear matrix DNA fraction and in bulk DNA.

The nuclear matrix DNA probe hybridized also intensely with the DNA retained in the pellet after hight salt treatment of nuclei followed by restriction endonuclease digestion (Fig. 2 f). This 'pellet-DNA' fraction has been considered by various authors to be enriched in nuclear matrix attachment sequences [e.g. 10,14,15]. This suggestion is supported by the results shown in Fig. 2 f. However, a significant amount of nuclear matrix DNA sequences appear also in the fraction of 'supernatant DNA' (Fig. 2 e). This shows that the recovery of nuclear matrix DNA sequences from nitrocellulose filters is more efficient than their recovery from 'pellet DNA'.

Discussion

The lower complexity of DNA retained on nitrocellulose filters compared to unfractioned DNA identifies the filter DNA fraction as a non-random sub-set of DNA sequences. Consequently, the tight DNA/ peptide complexes co-isolating with DNA and

inducing the binding of native DNA fragments to nitrocellulose filters are non-randomly associated with DNA. Moreover, the results shown here and previously [6,16, and preceeding paper] indicate that the tight DNA/ polypeptide complexes and the DNA sequences involved in such complexes reflect nuclear matrix components.

The homology between the DNA fractions investigated shows that the residual nuclear matrix DNA represents itself a sub-set of DNA sequences. This is of interest with respect to the significance of the DNA sequences retained in nuclear matrix prepared by the high salt procedure. The methods applied previously could not present clear evidence for the sub-set characteristics of nuclear matrix DNA (for discussion see [17]). Furthermore, it has been suggested that high salt treatment may destroy the physiological attachment sites of the genome at nuclear non-histone skeletons [18]. Our results can not rule out the possibility that high salt treatment may dissociate some interactions between distinct DNA sequences and nuclear non-histone components. However, it is highly unlikely that high salt treatment could accidentally induce the trapping of a clear sub-set of DNA sequences in the nuclear matrix. This suggests that the DNA sequences retained in the nuclear matrix and which are also comprised in the filter DNA fraction originate from sites where the genome is salt-stably interacting with non-histone components of nuclei.

Acknowledgment

This work was supported by a grant of the Deutsche Forschungsgemeinschaft (SFB 136, A3).

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