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# RADIOLABELLING OF DNA/ POLYPEPTIDE COMPLEXES IN ISOLATED BULK DNA AND IN RESIDUAL NUCLEAR MATRIX DNA BY NICK-TRANSLATION

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Conditions are described that allow $^{32}P$-radiolabelling and detection of tight complexes between DNA and polypeptides by nick-translation. Prolonged nick-translation of purified bulk DNA results in radiolabelled complexes migrating on SDS-polyacrylamide gels with apparent molecular weights of 68 kd and 54 kd respectively. Residual nuclear matrix DNA which is not accessible to DNase I on the nuclear level becomes accessible to radiolabelling by nick-translation on the nuclear matrix level. In this case the in situ radiolabelled complexes migrate on SDS-polyacrylamide gels with apparent molecular weights of 68 kd and 100 kd. The DNA/ polypeptide complexes are stable during treatments with SDS, ß-mercapto ethanol and alkali which points to covalent bonds between the polypeptides and DNA strands. 

DNA isolated by procedures considered to be efficient to purify DNA contains detectable polypeptide components. These polypeptides are not dissociated from DNA by high salt, SDS, proteases, phenol and alkali [1-8]. Even prolonged treatment of denatured DNA with high concentrations of proteases in the presence of SDS can not remove all peptides from DNA [5]. Previously, these tight DNA/ polypeptide complexes were detected by treatment of purified DNA with $^{125}$Iodine under conditions designed to iodinate proteins in tyrosine residues, heat degradation of the DNA, SDS-polyacrylamide gel electrophoresis and finally autoradiography [1,2,4,6]. According to such autoradiographs most of the protein-specific radiolabel remains at the top of the gels, which is due to irreversible aggregation of the proteinaceous components [3]. However, a portion of the radioiodinated polypeptides become solubilized in SDS-buffer and migrate on SDS-polyacrylamide gels with apparent molecular weights of 54 kd and 68 kd respectively. Although this method is sensitive and although 1 $OD_{260}$ unit of DNA is sufficient to detect polypeptides in DNA isolated from various organisms this method has been criticized [9]. It was suggested that ß-mercapto ethanol used to reduce the excess of $^{125}$Iodine may contain skin proteins which become radiolabelled under these iodination conditions [9]. Although we could not find such contaminations in accurately handled ß-mercapto ethanol and although the covalent nature of the bonds between DNA and radioiodinated peptides could be shown directly [5], the significance of the radioiodination

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procedure for the detection of DNA/ polypeptide complexes is still put into question. In this communication we shall describe a new technique for the radiolabelling and detection of tight DNA/ polypeptide complexes which is independent of the iodination reaction and which clearly indicates that the polypeptides co-purifying with DNA are intrinsically associated with DNA.

## *Methods*

Isolation of DNA: DNA was isolated from Ehrlich ascites cells by the procedure described by Gross-Bellard et al. [10]. This procedure involves lysis of cells in SDS buffer, digestions with proteinase K, ribonucleases and repeated phenol extractions.

Nuclear matrix of Ehrlich ascites cells: Nuclear matrix was prepared from nuclei of cells harvested at day 7 after inoculation according to a procedure published previously [7,11]. Briefly, nuclei were digested with protease-free DNase I (Boehringer, F.R.G.) which was followed by extractions with low-salt buffer (3 x) and high-salt (2M NaCl) buffer (3 x). Finally the nuclear matrices were washed once with low-salt buffer containing 1% Triton X-100 and suspended in low-salt buffer. All steps were performed at 4°C. The characteristics of the isolated nuclear matrices have been described elsewhere [7,11]. The fraction of residual DNA in our nuclear matrix preparations was previously found to be in the order of 1% of genomic DNA. The total amount of DNA in this cell type was found to be 11.59 $\mu$g/ $10^6$ cells [12].

Radiolabelling of DNA by prolonged nick-translation: The nick-translation reaction was performed by means of the nick-translation kit N 5000 (Radiochemical Center, Amersham, U.K.). Bulk DNA (8 $OD_{260}$ units) or a pellet of 5 x $10^6$ nuclear matrices (containing a total of 0.6 $\mu$g of DNA) were suspended in nucleotide buffer containing 100 $\mu$Ci of deoxycytidine 5'-[$\alpha$-$^{32}$P]triphosphate (3000 Ci/mmole) and optimal concentrations of DNase I and E.coli polymerase I. The reaction mixture was kept at 15°C for 18 hours. Protease-free DNase I (25 units) was added and the incubation was continued at 20°C for 30 min.

Isolation and detection of radiolabelled complexes in bulk DNA: Nick-translation assays were stopped by addition of 1 vol of 10% SDS solution containing 10% ß-mercapto ethanol and passed through a Sephadex G-25 column (10 ml) equilibrated with the following buffer: 150 mM NaCl, 0.5% SDS, 10% ß-mercapto ethanol, 2 mM EDTA and 25 mM Tris-HCl, pH 7.5. The same buffer was used for elution. Samples of 400 $\mu$l were collected and counted for radioactivity. The first three fractions containing radioactivity were pooled and precipitated with 7 vol acetone. The pellet was redissolved in 10% SDS/ 10% ß-mercapto ethanol and submitted to a new column as described above. After three cycles the first two fractions containing radioactivity were precipitated with acetone (7 vol). The pellets were redissolved in sample buffer [13] and analysed by SDS-polyacrylamide gel electrophoresis [13]. Gels were stained, destained, dried and exposed by standard procedures.

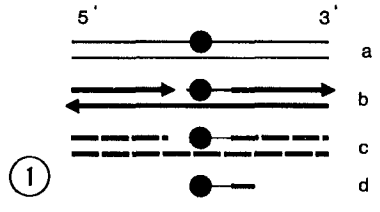
Isolation and detection of radiolabelled complexes in residual nuclear matrix DNA: The nick-translation reaction was stopped and passed through a Sephadex column as described above. Fractions containing the high molecular weight material were pooled, divided into three equal portions and precipitated with 7 vol acetone. One pellet was submitted to SDS-polyacrylamide gel electrophoresis without further treatments. The second pellet was first extracted with 1 ml 10 mM EDTA, 10 mM Tris-HCl, pH 8.0 containing 10% ß-mercapto ethanol and then submitted to SDS-polyacrylamide gel electrophoresis. The third pellet was treated with 0.5 M NaOH (20°C, 60 min), again precipitated and submitted to SDS-polyacrylamide gel electrophoresis.

## *Results*

### *Detection of DNA/ polypeptide complexes in isolated bulk DNA blocking nick-translation*

According to the mechanism of the nick-translation reaction a newly synthesized DNA strand growing into the direction of a blocking complex should remain disconnected from the complex by a nick between the 3'-end of the growing strand and the complex (Fig. 1b). However, DNA strands growing in the same direction but starting at induced nicks beyond the complex can be expected to remain linked to the complex (Fig. 1b). Finally, during prolonged incubations the deoxynucleoside triphosphates become used up and the DNase I in the reaction mixture shortens the DNA strands (Fig. 1c) leaving a portion of the radiolabelled oligo deoxy-nucleotides attached to the complex (Fig. 1d).

The nick-translation assays performed with bulk DNA had to be passed through three sequential Sephadex columns to remove most of the radiolabelled DNA degradation products overshadowing distinct bands in the autoradiographs of SDS-polyacrylamide gels. After three cycles two distinct bands could be resolved reflecting polypeptides with apparent molecular weights of 68 kd and 54 kd respectively (Fig. 2). Since DNA



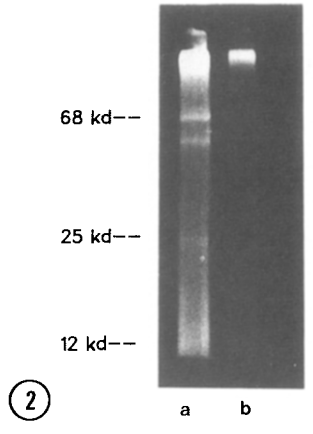
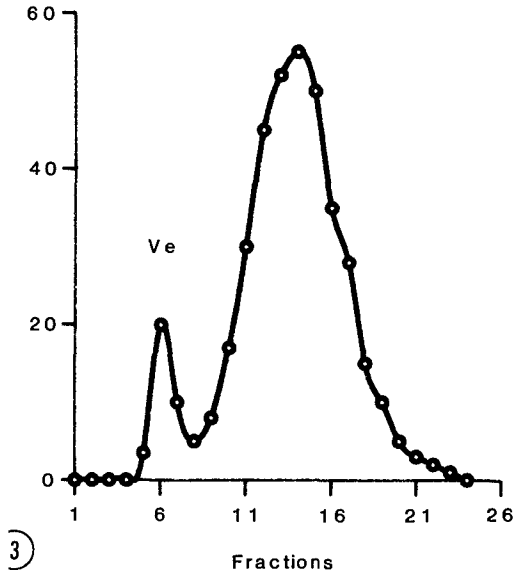




*Figure 1.* Schematic diagram illustrating the radiolabelling of DNA/ non-DNA complexes blocking the nick-translation reaction. Heavy lines represent radiolabelled DNA strands and segments. (a) DNA fragment associated with a presumptive complex (●) that renders the DNA strand inaccessible to E.coli polymerase I. (b) DNA strands growing into the direction of a complex become disconnected from the complex while DNA strands starting at the 3' side of a complex remain attached to complexes. (c) During prolonged nick-translation the DNA strands become increasingly degraded. (d) Finally, only small pieces of radiolabelled DNA may remain attached to the complex.

*Figure 2.* Autoradiograph of an SDS-polyacrylamide gel loaded with the high molecular weight material after the third Sephadex column chromatography of a prolonged nick-translation reaction performed with bulk DNA. Lane (a) represents the first, lane (b) the second fraction of the exclusion volume. The distinct bands on lane (a) reflect the radiolabelled DNA/ polypeptide complexes resolved from 130 $\mu$g of bulk DNA.



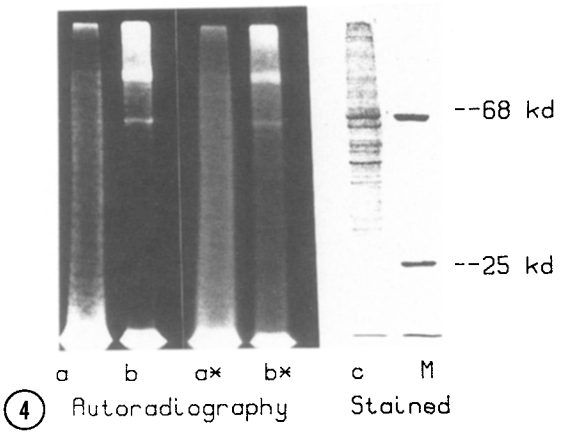




*Figure 3*, Sephadex column chromatography of nuclear matrix radiolabelled by prolonged nick-translation. Nuclear matrices were lysed after prolonged nick-translation and passed through a Sephadex G-25 column. 8.5% of the radiolabel appears in the exclusion volume (Ve) which was further analysed by SDS-polyacrylamide gel electrophoresis (Figs. 4 and 5).

*Figure 4*. SDS-polyacrylamide gel electrophoresis of radiolabelled DNA/ polypeptide complexes contained in the exclusion volume after Sephadex column chromatography of nuclear matrix submitted to prolonged nick-translation. Lanes a and a* without further treatments. Lanes b and b* after precipitation, extraction of soluble DNA and electrophoresis of the pellet solubilized by sample buffer. Autoradiography of a gel exposed for different periods (a/b and a*/b*). Lane c is identical with lanes b/b* but stained with Coomassie blue. Lane M reflects a stained track containing marker polypeptides.

polymerase I and DNase I were the only enzymes present in the reaction mixture, the polypeptides 68 kd and 54 kd in size are considered to be radiolabelled by the mechanism explained in Fig. 1. The same characteristic pattern of polypeptides has been shown previously by radioiodination of bulk DNA from various sources under conditions designed to iodinate polypeptides in tyrosine residues [1,2,4,6].

### *Detection of DNA/ polypeptide complexes in residual nuclear matrix DNA blocking nick-translation*

The nuclear matrix DNA which resists prolonged treatment with DNase I on the nuclear level is accessible to in situ radiolabelling by nick-translation on the nuclear matrix level. This may be due to the extraction of DNA-protecting nuclear proteins during the nuclear matrix isolation procedure. By incubation of isolated nuclear matrix with E.coli DNA polymerase I / DNase I and deoxycytidine 5'-[$\alpha$-$^{32}$P]triphosphate the radioactivity is incorporated at high rate into the residual nuclear matrix DNA strands.

Sephadex column chromatography of nuclear matrix submitted to prolonged nick-translation shows that a small but significant amount of $^{32}$P-label is eluted together with the high molecular weight material in a distinct peak (Fig. 3). Direct analysis of the

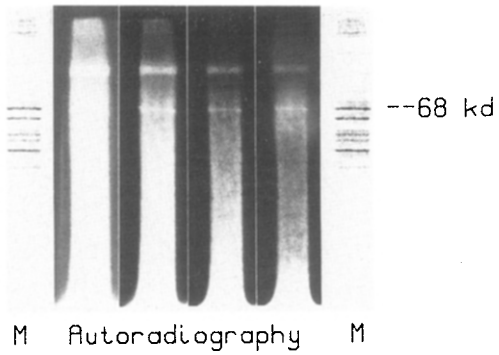



*Figure 5.* SDS-polyacrylamide gel electrophoresis of the radiolabelled materials contained in the exclusion volume (see Fig. 3) after alkali treatment. The gel was exposed for various times. Lanes M represent the same gel track after staining with Coomassie blue.

material in this peak by SDS-polyacrylamide gel electrophoresis gives no indication for a stable attachment of radiolabelled DNA strands to a non-DNA material (Fig. 4, lanes a and a*). The expectedly small amount of radiolabel intrinsically bound to polypeptides is overshadowed by the radiolabel in DNA strands which are not bound to polypeptides but which are still large enough to appear in the exclusion volume. Extractions of the acetone-precipitated material in the exclusion volume with low salt buffer releases a fraction of the DNA not stably attached to the insoluble pellet of nuclear matrix proteins. Analysis of the remaining portion of the radiolabel in the pellet of the low salt insoluble nuclear matrix polypeptides by SDS-polyacrylamide gel electrophoresis shows that a fraction of the radiolabel incorporated into DNA migrates like two polypeptides with apparent molecular weights of 68 kd and 100 kd respectively (Fig. 4, lanes b and b*). Since the pattern of the radiolabel is not coincident with the pattern of the major nuclear matrix polypeptides visualized on the same tracks by Coomassie blue staining this indicates that the $^{32}P$-label migrating in distinct bands on gels is neither trapped by polypeptides nor otherwise unspecifically transfered to polypeptides. Consequently, it can be concluded that at least a fraction of residual nuclear matrix DNA radiolabelled by nick-translation is tightly associated with nuclear matrix polypeptides. The bonds between this DNA fraction and the polypeptides resist prolonged treatments with SDS and ß-mercapto ethanol, they are stable during SDS-polyacrylamide gel electrophoresis and they are also not dissociated by treatment with a combination of alkali and ß-mercapto ethanol (Fig. 5).

## *Discussion*

The results show that bulk DNA and nuclear matrix DNA are associated with distinct polypeptides which are able to block the nick-translation reaction and which become radiolabelled during this procedure. The molecular weights of the DNA/ polypeptide complexes found in bulk DNA are identical in size with those which can be detected by

radioiodination of DNA [1,2,4,6]. This indicates that the polypeptides which can be detected by radioiodination of DNA are not artefacts due to contaminations present in ß-mercapto ethanol as suggested previously [9]. The DNA/ polypeptide complexes detected in residual nuclear matrix DNA show very similar physico-chemical characteristics as those detected in bulk DNA. They remain stably associated with DNA during treatments with SDS, ß-mercapto ethanol and alkali.

The polypeptide 68 kd in size is found in bulk DNA and in residual nuclear matrix DNA. The intensities of the autoradiographic signals corresponding to this complex are about equal in 0.2 µg of residual nuclear matrix DNA and in 130 µg of bulk DNA. This indicates that this complex is highly enriched in the nuclear matrix. The 100 kd polypeptide tightly associated with DNA is only found in residual nuclear matrix DNA while bulk DNA is associated with 54 kd polypeptides. It remains open whether the 100 kd polypeptide is only detectable when enriched in the 1% of genomic DNA retained in the nuclear matrix while it escapes its detection in bulk DNA or whether it is somehow degraded during the isolation of bulk DNA to result in complexes travelling on gels with an apparent molecular weight of 54 kd.

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