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# Nuclear matrix and chromosome scaffold preparations of in vitro cultured bovine liver cells have two proteins in common

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Nuclear matrices and chromosome scaffolds of in vitro cultured bovine liver cells were prepared under conditions that preserve the specific binding of the DNA. Protein compositions were analysed by electrophoresis and peptide mapping. Two slightly acidic polypeptides of apparent molecular masses 47 and 53 kDa were present in nuclear matrix as well as chromosome scaffold preparations. The corresponding matrix and scaffold proteins had identical peptide maps. Their putative function in the spatial organization of the DNA during the cell cycle is considered.

Nuclear matrix; Chromosome scaffold; Protein; (Mammalian cell culture)

## 1. INTRODUCTION

Studies on the organization of the nuclear DNA during replication and mitosis have supported the conception that a specific protein structure plays an important role in these processes [1–3]. There is evidence that nuclei and chromosomes contain a protein skeleton, resistant to treatment with 2 M NaCl, to which the DNA is bound by the origins of replication [2,4–6]. The salt resistant nuclear protein structure is usually called the nuclear matrix [7,8]. Its functional equivalent in mitotic cells is the chromosome scaffold [9,10]. We have previously reported that both nuclear matrices and chromosome scaffolds possess, among others, three proteins of the same electrophoretic mobilities, their relative molecular mass being 47, 53 and 71 kDa [11]. Here we show by peptide mapping that the 47 and 53 kDa proteins of chromosome scaffolds and nuclear matrices are identical.

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## 2. MATERIALS AND METHODS

### 2.1. *Preparation of nuclear matrices and chromosome scaffolds*

Nuclei and chromosomes were isolated from in vitro cultured bovine liver cells as described in detail elsewhere [11]. The isolated organelles were digested for 30 min at 0°C with 50 U/ml *Micrococcus* nuclease in Tris buffer, pH 8, containing 0.1 mM $CaCl_2$. An equal volume of 4 M NaCl in Tris buffer was then added and the residual protein structures were collected by centrifugation. The sediment was washed once with 2 M NaCl/buffer, once with plain buffer and once with 96% ethanol [11].

### 2.2. *Gel electrophoresis*

One-dimensional SDS-slab gel electrophoresis was carried out according to Laemmli [12] using 10% polyacrylamide gels. For two-dimensional electrophoresis [13] the samples were dissolved in 9 M urea/5% $\beta$-mercaptoethanol. Isoelectric focusing was performed in 4% polyacrylamide disc gels for 16 h at 400 V and another hour at 700 V, using a pH gradient from 5.5 to 9. The gels were



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washed in 50 mM Tris-HCl, pH 6.8. Separation in the second direction was carried out in 6–18% polyacrylamide gels in the presence of 0.1% SDS.

### 2.3. *Peptide mapping*

One-dimensional peptide mapping of polypeptides separated on 10% SDS-polyacrylamide gels was performed as described by Cleveland et al. [14] with slight modifications. The gels were fixed and stained with 0.1% Coomassie blue in 50% methanol/7% acetic acid overnight, destained in 20% methanol/7% acetic acid and rinsed in distilled water for 24 h. The gels were finally silver stained according to Eschenbruch and Bürk [15].

## 3. RESULTS

### 3.1. *Electrophoretic mobilities of residual nuclear proteins in 10% polyacrylamide gels*

Our previous studies have shown that nuclear matrix and chromosome scaffold preparations possess different sets of proteins [14]. However, electrophoretic mobilities of three polypeptides coincided, when both sets were compared in 6–18% polyacrylamide gels.

To characterize further these proteins by peptide mapping, they were first separated on 10% polyacrylamide gels. Fig.1 shows the patterns of a nuclear matrix preparation with lamins LA, LB and LC as major proteins and of a chromosomal scaffold preparation with an 83 and a 37 kDa polypeptide as major specific components. It is interesting to notice that some of the polypeptides differed in their mobilities relative to each other as compared to those in a 6–18% gel system. For example, a scaffold band (d) moved markedly slower than LC in the 10% gel but was slightly faster than the lamin in the gradient gel [11]. Furthermore, there was a distinct band (C) corresponding to the 71 kDa scaffold polypeptide reported in our previous paper, but its counterpart in the matrix (c) was poorly recognizable. Therefore, it is difficult to decide whether it represents the 71 kDa polypeptide, or a polypeptide which had moved slightly faster than lamin LB in the 6–18% gel [11], or both of them. On the other hand the 47 and 53 kDa scaffold polypeptides (f and e) coincided precisely with the corresponding matrix components (F and E) in both gel systems. This was also the case with a 45 kDa polypeptide (g and G),

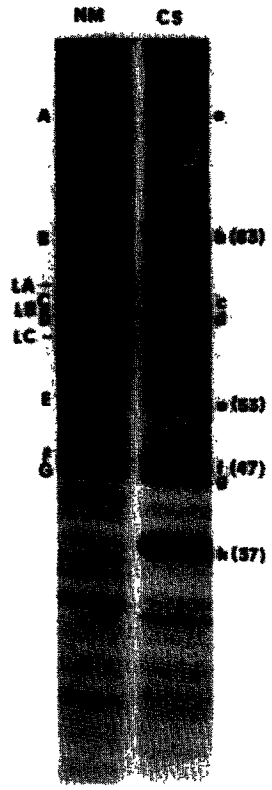


Fig.1. Electrophoresis of nuclear matrix and chromosome scaffold proteins in 10% polyacrylamide gels. 2 M NaCl resistant residual protein structures were prepared from isolated nuclei (NM) and chromosomes (CS) and separated by electrophoresis on 10% polyacrylamide gels in the presence of SDS. Polypeptides indicated by letters have been further analysed by peptide mapping. LA, LB and LC are the nuclear lamina proteins; numbers in brackets represent apparent molecular masses in kDa.

which has been shown to have the same electrophoretic mobility as cytoplasmic actin [11]. Actin has been reported to be associated with nuclear matrix preparations [16]. However, in view of strong quantitative variations it is difficult to judge to which extent it originates from nuclei or from cytoplasmic contamination.

We found no significant amounts of polypeptides Sc1 and Sc2 reported by Lewis and Laemmli [17]. This is due to the omission of the pretreatment with $Cu^{2+}$ which has been shown to be required for the presence of these proteins in scaffold preparations. We cannot exclude however

that bands a and A are residual traces of one of them.

### 3.2. *Peptide mapping*

Peptide maps were obtained of all bands indicated in fig.1 in order to find out (i) whether proteins with the same apparent molecular masses are identical, and (ii) if proteins with similar electrophoretic mobilities are related, i.e. modified forms of the same polypeptide. Fig.2 shows that peptide patterns of each of the 53 (E and e), 47 (F and f) and 45 (G and g) kDa polypeptides obtained from nuclear matrices are identical with the maps of the corresponding polypeptides of the chromosome scaffolds. We failed to obtain distinctive peptide maps of the high molecular mass polypeptides a and A by our analysis procedure.

No similarities of peptide patterns were found in any other pair of matrix and scaffold proteins. A typical example is given in fig.3, showing maps of several polypeptides from the 64–71 kDa range. None of the scaffold polypeptides mapped similar to any of those of the nuclear matrix.

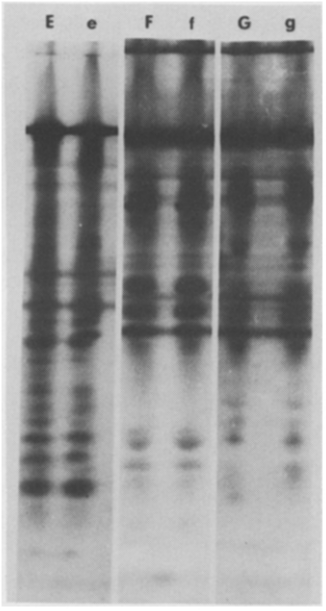


Fig.2. Comparison of peptide maps of polypeptides with identical electrophoretic mobilities. Lanes E, F and G are of nuclear matrix, e, f and g of chromosomal polypeptides (see fig.1).

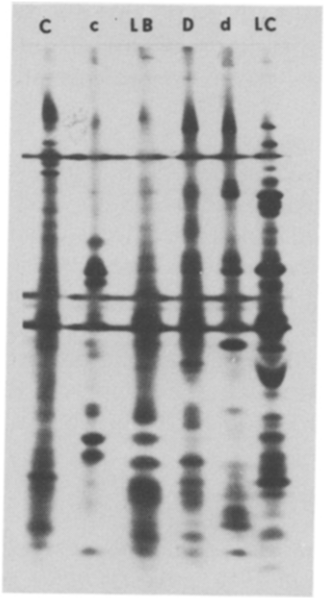


Fig.3. Comparison of peptide maps of polypeptides with slightly different electrophoretic mobilities. Lettering refers to the polypeptide bands indicated in fig.1.

### 3.3. *Two dimensional gel electrophoresis*

Matrix and scaffold polypeptides were further characterized by two-dimensional electrophoresis according to O'Farrel [13]. The separation pattern of the matrix proteins in fig.4a shows the well known distributions of lamins LA, LB and LC [18]. The majority of the minor polypeptides had moved towards the acidic range (see also [3]). The 47 and 53 kDa polypeptides focused as single spots in the neighbourhood of LB. Considering a p*I* of 6 for LB [18], the p*I* values of the 47 and 53 kDa polypeptides are estimated to be 6.2 and 5.9, respectively. Essentially all polypeptides of the chromosomal scaffolds focused in the acidic range (fig.4b). The 47 and 53 kDa polypeptides are found at the same positions as in fig.4a. The major specific scaffold components (37 and 83 kDa) extended over a broad pH range suggesting that they occur in several isoelectric forms. Average p*I* values were 6.2 for the 37 kDa and 6.6 for the 83 kDa polypeptide. It might be mentioned that traces of these polypeptides are also found in matrix preparations (fig.4a). We ascribe this to the fact that nuclear preparations contain substantial proportions of prophase nuclei in which the rear-

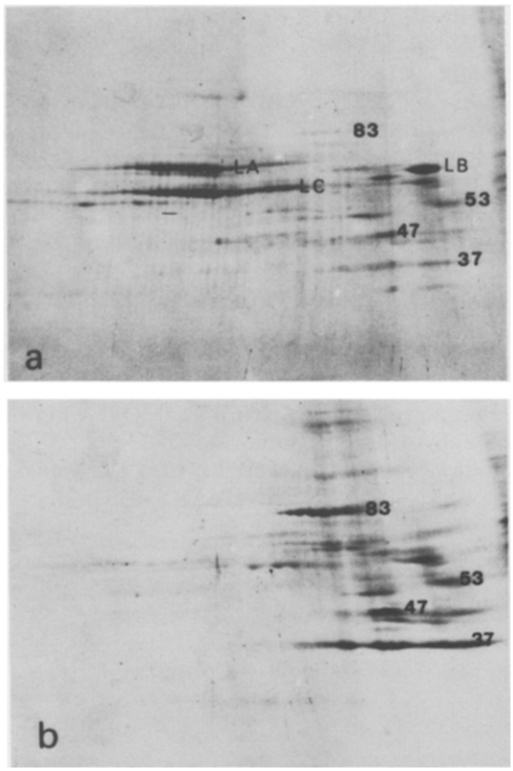


Fig.4. Two-dimensional electrophoresis of nuclear matrix and chromosome scaffold proteins. 2 M NaCl resistant residual protein structures were prepared from isolated nuclei (a) and chromosomes (b) and separated by two-dimensional electrophoresis according to O'Farrel [13]. LA, LB and LC are lamina proteins. Numbers indicate apparent molecular masses in kDa of the spots to their left. Isoelectric focusing was from left (basic) to right (acidic).

rangement of the nuclear matrix into chromosomal scaffolds is already in progress.

## 4. DISCUSSION

We have isolated a residual nuclear structure under conditions that yield a minimal number of proteins but warrant a reproducibly specific binding of the DNA. When nuclei or chromosomes are extracted with 2 M NaCl, DNA is found to be attached to the residual structures by the origins of replication and, during replication, by the replication forks ([2,4–6,19,20] and many others). The residual structures, nuclear matrices and chromosomal scaffolds, have very different compositions of mainly acidic proteins, but both contain reproducibly 2 polypeptides of 47 and 53 kDa [11]. The results of peptide mapping and two-dimensional electrophoresis presented here indicate that the two corresponding pairs of proteins in matrix and scaffold preparations are identical.

Therefore, the 47 and 53 kDa polypeptides can be considered to be the essential components of the backbones to which the DNA is bound in interphase nuclei as well as in chromosomes [2,3]. From the staining patterns we estimate that they represent between 0.1 and 1% of the total nuclear protein content. Assuming an average of 0.5% and a nuclear DNA/protein ratio of 0.5 one can calculate that there is one protein of 50 kDa per $5 \times 10^3$ kDa DNA, which is equivalent to about $8 \times 10^5$ base pairs. This means that there are about 10 polypeptides of both kinds per replication origin at an average replicon size of $10^3$ base pairs.

Thus we envisage these two proteins as elements of the filamentous structures to which the DNA is bound by the replication origins throughout the cell cycle [2–5]. The cause for the folding up of the irregularly wound interphase filaments into solenoid-like scaffolds during chromosome condensation is not clear. Protein modification might be one possibility. However, peptide maps and two-dimensional electrophoretic patterns give no indication of modifications of the two proteins. Another possibility for the reorganization is the addition of further proteins. Such a function could reside in one or more of the polypeptides that are specific for either nuclear matrix or chromosomal scaffold preparations. We would like to mention that chromosome lengths increase almost 10-fold in the presence of 2 M NaCl (unpublished). This means that additional, saline soluble proteins are required or/and that ionic interactions also play a role in the regular folding up.

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