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Expression and characterization of human lamin C

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We have expressed human lamin C cDNA in *E. coli* using a modification of the pLcII vector system. Protein produced in this way had seven additional amino acids at its N-terminus, but retained key lamin structural and assembly properties. The modified vector we produced may prove useful when difficulties are encountered in removal of the cII fusion peptide by factor X cleavage in the pLcII system. Shadowed preparations of expressed lamin C showed the presence of 50-nm rod-like particles that closely resembled those observed for native material. Isolated molecules had two globular domains at one end, indicating that they were constructed from two parallel polypeptide chains. The expressed material also formed paracrystals with a characteristic 22.5 nm axial repeat, indicating that its assembly properties had also been retained. We also used site-specific mutagenesis to engineer a lamin fragment that lacked the C-terminal non-helical domain of the molecule. This material formed paracrystals similar to those obtained with the intact molecule, indicating that the large C-terminal non-helical domain did not contain information vital for lamin assembly.

Nuclear envelope; Mutagenesis; Paracrystal; Assembly

INTRODUCTION

Lamins are the principal component of the fibrous lamina that underlies the nuclear envelope of eukaryotic cells [1]. The lamina fibres are about 10 nm in diameter and are arranged in a dense fibrous mat that sometimes shows a remarkable tetragonal order [2,3]. The lamina is thought to be important in organizing interphase chromatin and in maintaining nuclear envelope integrity [4]. Mammals express three lamin isoforms (A, B and C); lamins A and C are very similar and appear to arise by alternative splicing [5,6]. Although B-type lamins appear to be ubiquitous, lamins A and C are usually expressed only in differentiated cells [7]. Lamin sequences [5,6] show strong homologies to intermediate filament (IF) proteins and indicate a three-domain structural model with a central fibrous rod, having principally an α-helical-coiled-coil conformation, flanked by non-helical N- and C-terminal domains (see [8,9]). The lamin N-terminal domain is small compared with most other IF proteins, whereas the lamin C-terminal domain is comparatively large. Electron microscopy of shadowed molecules and paracrystals [2,10] support this model and show a rod-shaped molecule, about 52 nm long, with two prominent globular domains at one end. In vivo studies have identified a putative lamin nuclear localization signal and have also indicated a role for part of the C-terminal domain in the proper assembly of the nuclear lamina [11]. Several mutants in which portions of this domain were deleted still localized to the nucleus, but there formed aberrant structures. Clearly some knowledge of lamin structure and the molecular basis for assembly into filaments and binding to components of the nuclear envelope is required to understand the role of the lamina in nuclear envelope dynamics and chromosome organisation. Because the cellular volume fraction of nuclear envelope is small, difficulties can be experienced in preparing large quantities of lamins necessary for studying their assembly properties and structure. Moreover, native material usually is phosphorylated to some extent and this can complicate the assessement of assembly properties. Recently several IF protein cDNAs have been expressed in *E. coli[[12,13] enabling large quantities of material to be produced. Moreover, expression in E. coli* facilitates the production of fragments and modified material using site-specific mutagenesis, which can be used to dissect the molecular basis of assembly and domain structure and so complement expression studies in cultured cells. We have therefore developed a system to enable lamins to be produced in this way and describe here the production of substantial quantities of human lamin C. This material closely resembled native lamin in its assembly properties and ability to form ordered paracrystals. We have also deleted the C-terminal non-helical domain of the molecule and find that fragment forms paracrystals similar to those obtained with whole lamin C.

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MATERIALS AND METHODS

Manipulations of DNA, site-specific mutagenesis, and bacterial strains were as described [13]. Human lamin C cDNA was the gift of Dr. F. McKeon (Harvard University). Oligonucleotides were synthesised by Terry Smith (MRC Laboratory of Molecular Biology, Cambridge) using an Applied Biosystems synthesiser. The construct we used to express human lamin cDNA is illustrated in Fig. 1. We also used oligonucleotide-directed site-specific mutagenesis to delete the DNA coding for the C-terminal non-helical domain. A 33-mer was used to move the stop codon to immediately after base 1179 so that the expressed protein was truncated at Pro-393. Proteins were expressed as described [14] and were located in inclusion bodies. When inclusion bodies were isolated as described [14], the expressed protein was degraded rapidly. Therefore, to minimise proteolysis, we sonicated bacterial pellets directly in 8 M urea, 20 mM Tris-HCl, 1 mM EDTA, 1% β-mercaptoethanol, pH 8. Insoluble material was removed by centrifugation at 70 000 × g for 30 min and the supernatant applied to 15 × 1.5 cm TSK CM-650(M) column in sonication buffer. The column was developed with a 0–500 mM NaCl gradient. Sometimes the protein was purified further using a Pharmacia FPLC system and Mono S column developed with sonication buffer. Protein was then frozen in this solvent until required. The protein was renatured by dialysis against 4 M urea, 1 mM EDTA, 2 mM dithiotheitol (DTT), 20 mM Tris.HCl, pH 8, at room temperature for 1 h and then exhaustively against 250 mM NaCl, 2 mM DTT, 20 mM Tris.HCl,pH 8 at room temperature.

SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli [15]. Protein concentration was determined by the Bradford [16] method. Western blotting was carried out as described [13], using rabbit polyclonal antibody directed against rat lamins (which was the generous gift of Dr Larry Gerace, Scripps Clinic, La Jolla). For electron microscopy, isolated particles were sprayed onto mica in 50% glycerol, 250 mM NaCl, 20 mM Tris.HCl, pH 8, and shadowed in a Cressington CFE-50 system with platinum-carbon as described [17]. Paracrystal suspensions were prepared as described [2] and applied to carbon-coated 400-mesh electron microscopy grids and negatively stained with unbuffered 1% aqueous uranyl acetate as described [18]. Both negatively stained and shadowed specimens were examined at 80 kV in a Philips EM400 electron microscope using standard conditions. Microscope magnification was calibrated by reference to negatively-stained sheaths of *Methanospirillum hungatei* [19].

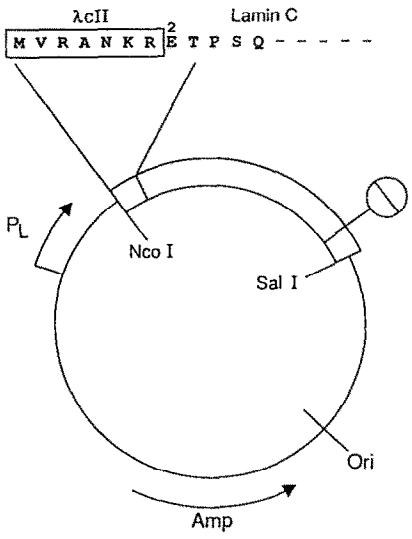

Fig. 1. Representation of the pLcII plasmid used for the expression of human lamins in *E. coli*. The pLcII vector system has been described in detail elsewhere [14]. The portion of the vector coding for the cII protein was truncated to 21 bases (coding for the N-terminal 7 amino acids) then fused to cDNA coding for full-length human lamin C [6], starting at the second codon, using oligonucleotide-directed site-specific mutagenesis [13]. The fusion gene was under control of the P_L promotor and coded for a protein containing 578 amino acid residues. In addition, a mutant protein, corresponding to the lamin C rod and N-terminal non-helical domain, was produced by truncating the lamin cDNA and introducing a stop codon after base 1179, corresponding to Pro-393.

RESULTS

We have produced substantial quantities of recombinant human lamin C by expressing cDNA in *E. coli* under the control of the λ P_L promotor. High levels of expression were obtained the pLcII vector system [14], in which the required protein is synthesised as a fusion protein with the first 32 residues of the λ cII protein. By engineering a factor X proteolysis site after the λ cII peptide, the required material can be subsequently obtained by proteolysis. However, removal of the 32 residual of the λ cII protein using factor X was not possible without substantial proteolysis of the lamin polypeptide chain, even though the lamin C sequence did not contain the consensus for factor X cleavage. Cleavage with thrombin, which has sometimes been used in these circumstances [13] was also unsuccessful. We therefore experimented with modifications of the pLcII vector ystem in which we deleted portions of the cII sequence to see if it was possible to obtain a fusion protein in which such a small quantity of the cII protein remained that it did not interfere with the structure and assembly of the expressed protein. Although almost negligible amounts of expression were obtained when the entire cII sequence was deleted (data not shown), quite high levels were obtained when only the first 7 residues (including the initiator Met) were used and the native initiator Met of the lamins was deleted. We obtained about 15 mg of lamin C per litre of culture in this system, approximately half of that using the full cII fusion protein. Although we have not fully explored the minimum requirements for efficient expression in this system, it is likely that a heptamer with dyad symmetry (AAACAAA) spanning codons 4–6 of the cII protein is important. Such an inverted repeat may, for example, function as a nucleic acid-protein interaction site in transcription or translation.

Polyacrylamide gel electrophoresis (Fig. 2) indicated that the fusion protein derived from lamin C, had a M_r of approximately 63 000 which was in good agreement with the value of 65886 Da predicted from the sequence [5,6]. We also used site-specific mutagenesis to produce a construct corresponding to only the N-terminal non-helical domain and the rod. This molecule was truncated at Pro-393 and had a M_r of 40 000 (Fig. 2). Similar levels of expression were obtained as with the full length lamin C. Both the full-length lamin C and the mutant gave a positive reaction with an anti-lamin polyclonal antibody on Western blots.

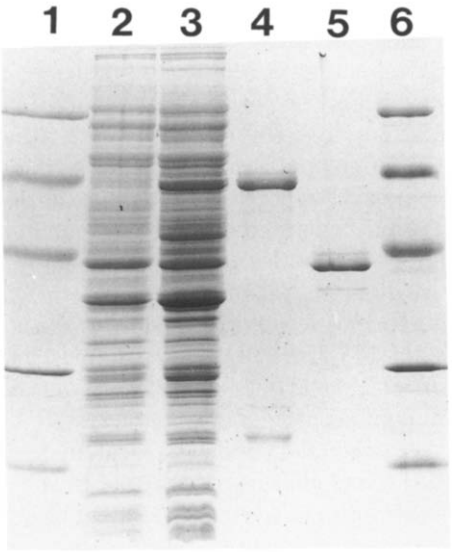

Fig. 2. SDS-polyacrylamide gel showing expression of human lamins in the pLcII vector system. (Lanes 1 and 6) M_r markers, phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa); (Lane 2) whole cell lysate before induction by heat shock; (lane 3) whole cell lysate after induction; (Lane 4) purified lamin C after ion exchange chromatography; (lane 5) purified lamin fragment containing the N-terminal non-helical domain and coiled-coil domain (residues 2–393 [5,13].

Shadowed preparations of dilute solutions of the purified lamin C sprayed in glycerol onto mica showed distinctive rod-like molecular profiles consistent with the fibrous nature of lamins (Fig. 3). These rods generally resembled similarly shadowed preparations of native material [2] and, significantly, showed two globular domains at one end. The presence of these two globular domains at one end of the shadowed molecules indicated that they were constructed from two parallel chains as found in native material [2]. Areas in which the rods were fairly well separated were common and measurements of particle lengths gave a mean value of 50.4 nm (SD = 2.5, $n = 50$), which was not significantly different to the 52 nm reported [2] for a mixture of rat liver lamins A and C.

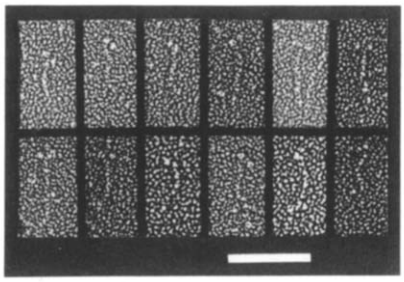

Fig. 3. Gallery of electron micrographs of expressed lamin C molecules rotary shadowed with platinum-carbon. The molecules have a characteristic rod shape with two globular domains at one end (oriented towards the top of the figure). Bar = 100 nm.

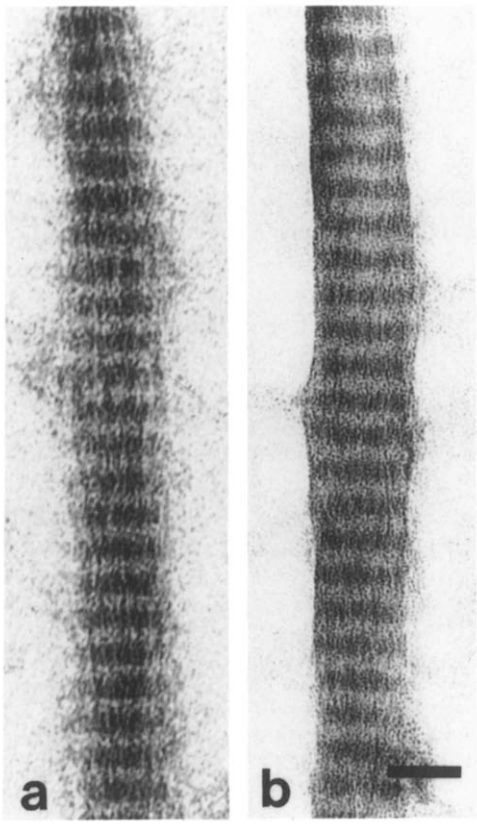

Fig. 4. Electron micrographs of paracrystals negatively stained with uranyl actetate. (a) Full length lamin C, (b) truncated lamin C fragment corresponding to the N-terminal non-helical domain and the coiled-coil rod. Although the contrast was higher in full-length lamin C paracrystals, the axial staining pattern observed with both was very similar and was characterized by an alternating series of light and dark bands with an axial repeat of 22.5 nm. Bar = 40 nm.

Lamins A and C are soluble at high (0.5 M) ionic strength [2] and our expressed lamin C was similarly soluble under these conditions. Moreover, when the ionic strength is reduced, native lamins form paracrystals [2] and we observed a similar phenomenon with the expressed lamin C (Fig. 4). Negatively-stained paracrystals had a characteristic 22.5-nm axial banding pattern of alternating light and dark bands that was identical to that seen with native material [2]. The lamin fragment corresponding to the rod and N-terminal domain also produced paracrystals with a similar axial repeat and pattern (Fig. 4).

DISCUSSION

By using a modification of the pLcII expression system, we have produced large quantities of human lamin C in *E. coli*. By deleting all but the first 7 residues of the λ cII protein that is fused to the expressed protein in this vector, we retained high levels of expression together with the convenience of induction and mutagenesis which characterise this system [14], while reduc-

ing the size of the fusion protein at the N-terminus of the material sufficiently so that it did not interfere with the structure and assembly properties of the protein. This may be a promising method for circumventing problems with this vector system that are often encountered when attempting to remove the cII fragment by proteolysis.

Although the material we produced using the modified pLcII vector had 7 extra amino acid residues located at its N-terminus, its structural and assembly properties were indistinguishable from those of lamins isolated from mammalian cell nuclear envelopes [2]. Direct observation of renatured expressed molecules by electron microscopy showed that they had recovered their characteristic rod-like structure with two prominent globular domains at one end [2]. Moreover, the assembly properties of the expressed material were preserved to the extent that we were able to produce characteristic paracrystals when the ionic strength was reduced. Since the molecular interactions in these paracrystals are thought to resemble closely those found in the lamina fibres in vivo [2,10] the formation of these paracrystals is a particularly stringent test that the structural and assembly properties of the native material were retained in the expressed lamin C.

Previous studies of lamin structure and assembly [2,10] have used a mixture of lamins A and C. Since these proteins are always expressed together, it was not clear if both isoforms had to be present either to form molecules or to assemble into higher aggregates as found, for example, in cytokeratins [8,9]. Our demonstration that intact molecules and also paracrystals can be obtained from only lamin C show that these assembly properties are possessed by a single isoform. Moreover, lamins isolated from mammalian cells have been subject to a number of post-transcriptional modifications including phosphorylation, isoprenylation and proteolysis [20,21]. Our demonstration of molecular assembly and paracrystal formation with unmodified expressed material indicated that these various post-transcriptional modifications are not required for these lamin interactions.

The paracrystals obtained from the lamin fragment from which the C-terminal non-helical domain had been removed were very similar to those obtained with full length material. Both paracrystals had an alternating pattern of light and dark bands with an axial repeat of 22.5 nm, although the contrast of the pattern was somewhat lower with the paracrystals formed from the mutant. The similarity of the two patterns indicated that the rod and N-terminal domains contained all the information required for paracrystal assembly and is consistent with observations on a range of intermediate filament proteins that indicated that the C-terminal domain was not vital for molecular interactions [8,9]. Also, it has been proposed [10] that the staining pattern observed with lamin paracrystals derived from stain exclusion by the large non-helical C-terminal domain. Clearly our observation of a similar staining pattern when this domain has been deleted indicated that such a stain-exclusion mechanism cannot account entirely for the pattern formed. Although such a mechanism may make some contribution to the staining pattern (and probably accounts for the higher contrast observed with the full-length lamin C paracrystals), it is likely that a major contribution to the pattern comes from the lamin rod domains forming a gap-overlap structure, analogous to that seen with other IF protein fragment paracrystals [22]. Since the length of lamin molecules is not an integral multiple of the 22.5 nm paracrystal repeat, the molecules in the paracrystals cannot be packed precisely end-to-end. The local thickening of the paracrystal where ends overlap would thus be expected to produce a white band (corresponding to greater density) and so account for the observed pattern.

The development of this expression system for human lamin C enables the production of large quantities of material to study lamin assembly and structure and will also enable the production of defined molecular fragments to test the role of the different structural domains of the molecule in assembly and in interaction with other components of the nuclear envelope, such as nuclear pores. Mutants that produce defined effects in vivo can also be assessed and characterised extensively in vitro to determine precisely how these mutations interfere with lamin structure and assembly.

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