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RPII15 codes for the M_r 15,000 subunit 9 of *Drosophila melanogaster* RNA polymerase II

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The *RPII15* gene product of *Drosophila melanogaster*, which has recently been identified by sequence comparison, possesses a high similarity to subunit 9 of yeast RNA polymerase II. Using the polymerase chain reaction the coding region of *RPII15* was isolated from genomic DNA of adult flies. Sequence analysis shows four amino acid substitutions in comparison to the previously reported sequence. Antisera were generated against bacterially expressed *RPII15* and were used for immunoblotting experiments with RNA polymerase II of *Drosophila melanogaster*. This analysis identified the M_r 15,000 subunit 9 as gene product of *RPII15*.

RNA polymerase II; Small subunit; Maltose-binding protein; Fusion protein; *Drosophila melanogaster*

1. INTRODUCTION

Eukaryotic nuclear RNA polymerases (E.C. 2.7.7.6.) consist of two large subunits with molecular weights between 140 and 220 kDa and about 9 to 13 smaller subunits with molecular weights between 10 and 80 kDa (for review see [1,2]). The genes for all subunits of yeast RNA polymerase II have been isolated and sequenced in the past years while only the genes for the largest (*DmRP215*) and second-largest subunit (*DmRP140*) of *Drosophila* RNA polymerase II have been cloned [3,4]. The gene encoding the 12.2 kDa subunit 9 (RPB9) of yeast RNA polymerase II has been isolated by screening of a plasmid library with a probe designed from sequenced peptides [5]. Sequence analysis showed that this subunit is similar to a *Drosophila* protein encoded by a gene upstream of the suppressor of Hairy wing (su(Hw)). cDNA of this gene (*RPII15*) was isolated which codes for 129 amino acids with a predicted molecular weight of 15,100 Da [6]. Detailed analysis of *RPII15* showed that this gene is expressed at all developmental stages and in all tissues and that it is essential for viability. However, at the moment it is not clear which of the smaller subunits of RNA polymerase II is encoded by this gene. To answer this question, we generated antibodies against recombinant *RPII15* and performed immunoblotting experiments to identify the subunit encoded by *RPII15*.

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Abbreviations: CTD, C-terminal heptapeptide repeat domain; MBP, maltose-binding protein, PBS, phosphate-buffered saline.

2. MATERIALS AND METHODS

2.1. *Materials*

RNA polymerase II was purified as described [7] except that the Superose-6 gel filtration step was omitted. Genomic DNA was isolated from adult flies according to [8]. Primer 1 (CTGGATCCGATATCATGACGACTGCCTTTGATGC) and primer 2 (CCAAGCTTCAGTTTCTACTCCGTCCAAC) were synthesized in the lab of Dr. R.W. Frank (ZMBH, Heidelberg). Tth DNA polymerase was purchased from Epicentre.

2.2. *Cloning and expression of RPII15 and production of antiserum*

The coding region of *RPII15* was amplified from genomic DNA by PCR (30 cycles, 1 min 95°C, 1 min 58°C, 1 min 72°C) using primer 1 (introducing an additional triplet at the 5′ end of the *RPII15* reading frame encoding an isoleucine) and primer 2 and the following reaction buffer (50 mM KCl, 13 mM $MgCl_2$, 200 μM dNTPs, 50 pM primer each, 2.5 U Tth DNA polymerase). The resulting 430 bp fragment was cloned into expression vector pMAL-c (Biolabs). DNA sequencing was performed using a T7 sequencing kit (Pharmacia). Expression and purification of recombinant fusion protein and cleavage with factor Xa was performed according to the manufacturer's protocol. For production of antisera New Zealand white rabbits were immunized 4 times with 300 μg purified fusion protein in intervals of 2 weeks using ABM-S (Linaris) as adjuvans and were finally bled 3 days after the last injection.

2.3. *Gel electrophoresis and immunoblotting*

Proteins were separated on 6–20% or 10–20% linear gradient polyacrylamide gels by SDS-PAGE [9] and were either stained with Coomassie brilliant blue R-250 or were transferred onto nitrocellulose sheets (Millipore) by semidry blotting (Pharmacia-LKB). Remaining binding sites were blocked with PBS pH 7.3, 0.3% Tween-20. Blotted proteins were incubated with anti-serum generated previously against purified RNA polymerase II (diluted 1:500 in blocking buffer) or with anti-serum generated against purified MBP-RPII15 fusion protein (diluted 1:100), respectively, for 2 h at room temperature. After washing, the filters were incubated for 1 h with peroxidase-conjugated goat anti-rabbit immunoglobulins (Dianova) diluted 1:2500. Bound antibodies were detected using diaminobenzidine/H_2O_2 as substrate.

3. RESULTS

3.1. *Isolation of the RPII15 coding sequence from genomic DNA*

The coding region of *RPII15* was amplified from genomic DNA of adult flies by polymerase chain reaction using primers corresponding to the 5′ and 3′ terminal regions of the *RPII15* coding sequence reported by Harrison and co-workers [6]. The polymerase chain reaction resulted in a single product of 430 bp. Sequence analysis of the deduced amino acid sequence revealed four amino acid substitutions in comparison to the reported sequence (Fig. 1). These changes residing at amino acid positions 51 (K-E), 52 (T-A), 53 (N-D), and 102 (K-Q) increase the similarity of *RPII15* to subunit 9 of yeast RNA polymerase II from 46% to 48% identical residues. Two further changes in the nucleotide sequence affected only the third position of the triplets and had no effects on the deduced amino acid sequence (Fig. 1).

3.2. *Expression and purification of MBP-RPII15 fusion protein*

The coding region was fused to maltose-binding protein resulting in a fusion protein with a molecular weight of 58 kDa as determined by SDS-PAGE analysis. The protein was purified by maltose affinity chromatography and the fusion protein was cleaved with factor Xa. As shown in Fig. 2 the RPII15 cleavage product has the expected size of 15 kDa.

3.3. *Reactivity of antiserum with subunit 9 of RNA polymerase II*

Analysis of *Drosophila melanogaster* RNA polymerase II by SDS-PAGE identifies 12 distinguishable subunits (Fig. 3). The RPII15 cleavage product comigrates with the 15 kDa subunit 9 (B9) of RNA polymerase II (Fig. 3). Antiserum generated against purified MBP-RPII15 fusion protein was used for immunoblotting experiments with cleaved fusion protein and with purified RNA polymerase II. The antiserum recognizes both the MBP and the RPII15 fusion part indicating that antibodies were generated against the RPII15 sequence. The serum also reacts with the 15 kDa subunit 9 of RNA polymerase II (Fig. 3). A mixture of two antisera generated previously against RNA polymerase II shows a strong reaction with subunits B1 (175 kDa IIb form lacking the CTD), B2 (140 kDa), B3 (34 kDa), B4 (27 kDa), B5 (21 kDa), and B10 (~ 13 kDa) and to a lesser extent with subunit B8 (18 kDa) and subunit B9 (15 kDa). No reactions were observed with subunits B6 (20 kDa), B7 (18.5 kDa) B11 (~ 12 kDa), and B12 (~ 11 kDa).

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  1 ATGACGACTGCCTTTGATGCCGCACACACTGAGGGGCCGGGATTCGTGGGCATTCGGTTC
  1  M  T  T  A  F  D  A  A  H  T  E  G  P  G  F  V  G  I  R  F

 61 TGCCAGGAGTGCAACAACATGCTGTACCCCAAGGAGGACAAGGAGAACAAGATCCTGCTG
 21  C  Q  E  C  N  N  M  L  Y  P  K  E  D  K  E  N  K  I  L  L

                                  *  *  *
121 TACGCCTGCCGGAATTGCGATTACAAACAGGAGGCGGACTCCAACTGCATCTACGTGAAC
 41  Y  A  C  R  N  C  D  Y  K  Q  E  A  D  S  N  C  I  Y  V  N

181 AAGATTATGCACGAGATCGACGAGCTGACCCACATTGTGCCCGACGTGATTTCCGATCCC
 61  K  I  M  H  E  I  D  E  L  T  H  I  V  P  D  V  I  S  D  P

                                              *
241 ACGCTGCCGCGCACCGAAGACCACGCTTGTCCCAAGTGCTCCCATCGGGAGGCGGTCTCC
 81  T  L  P  R  T  E  D  H  A  C  P  K  C  S  H  R  E  A  V  F

       *           *
301 TTCCAGGCGCAAACTCGTCGCGCCGAAGAGGAGATGCGACTGTACTACGTGTGCACCAAC
101  F  Q  A  Q  T  R  R  A  E  E  E  M  R  L  Y  Y  V  C  T  N

361 CAGAACTGCACCCACCGTTGGACGGAGTAGAAACTG
121  Q  N  C  T  H  R  W  T  E  *
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Fig. 1. RPII15 sequence of PCR amplified genomic DNA. The nucleotide sequence and the deduced amino acid sequence are shown. Nucleotides and amino acid residues which are different from the reported RPII15 sequence [6] are indicated by asterisks or bold letters, respectively.

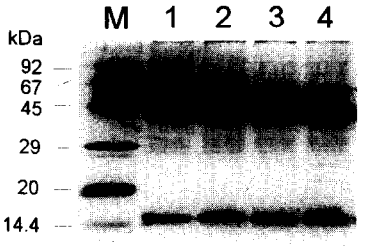

Fig. 2. Purification and cleavage of MBP-RPII15 fusion protein. One microgram purified MBP-RPII15 fusion protein was cleaved with factor Xa and samples were taken after varying times (lane 1, 2 h; lane 2, 4 h; lane 3, 15 h; lane 4, 24 h). Proteins were analyzed on a 15% polyacrylamide gel by SDS-PAGE.

4. DISCUSSION

This study showed that the *RPII15* gene previously identified by sequence similarity to a yeast RNA polymerase II subunit gene codes for a small subunit of *Drosophila* RNA polymerase II. As in yeast this gene encodes the ninth subunit of RNA polymerase II. The subunit possesses the same molecular weight (determined by SDS-PAGE analysis) as the amino acid sequence deduced from the *RPII15* reading frame. In contrast, subunit 9 of yeast RNA polymerase II has a predicted molecular weight of 14,200 Da but migrates as a 12.2 kDa protein in polyacrylamide gels.

The differences between the sequence of *RPII15* isolated in this study and the reported sequence points either to a polymorphism within the nucleotide sequence of the gene or to strain specific variations. Of the four amino acid differences deduced from our sequence analysis, three show identity with the yeast subunit. Therefore, it is unlikely that these observed changes are due to errors in the polymerase chain reaction and it is rather likely that our sequence is the correct one.

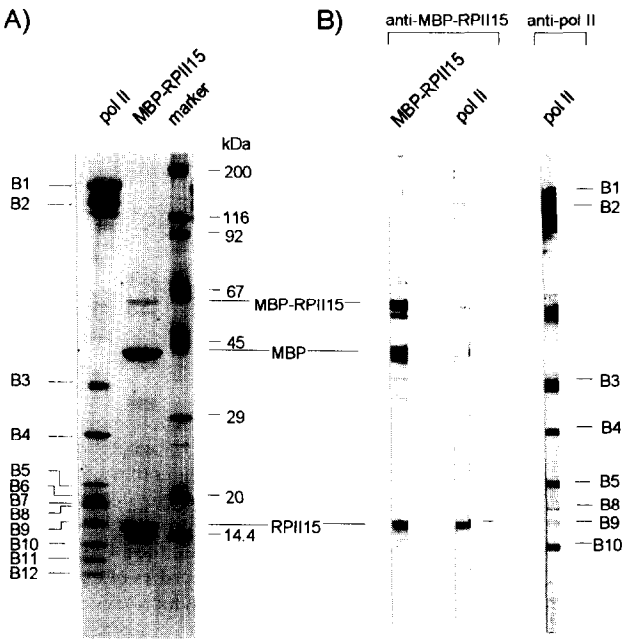

Fig. 3. Immunoblot analysis of RPII15 antiserum. (A) Purified *Drosophila* RNA polymerase II (5 μg) and MBP-RPII15 fusion protein (2 μg) cleaved with factor Xa for 1 day were separated on a 10–20% gradient polyacrylamide gel by SDS-PAGE. The positions of the 12 distinguishable subunits of RNA poymerase II (B1 to B12) with molecular weights between 175 kDa and ~10 kDa are indicated. The 175 kDa subunit represents the largest 215 kDa subunit lacking the CTD which is proteolytically cleaved during purification. (B) Immunoblotting experiments with antiserum generated against MBP-RPII15 fusion protein (anti-MBP-RPII15). The serum was incubated with cleaved MBP-RPII15 fusion protein or with RNA polymerase II (polII), respectively. In addition, the reactivity of a mixture of two sera against RNA polymerase II (anti-polII) with RNA polymerase II is shown.

The weak reactivity of polyclonal antibodies generated previously against native RNA polymerase II with subunit 9 in immunoblotting experiments led to the suggestion that this subunit is only weakly immunogenic or that the antigenic structure of this subunit disintegrates in western blotting.

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