Structural and functional similarities between HIV-1 reverse transcriptase and the *Escherichia coli* RNA polymerase β′ subunit

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Abstract Four monoclonal antibodies (MAbs) recognizing HIV-1 reverse transcriptase (RT) were shown here to cross-react with the β′ subunit of *Escherichia coli* RNA polymerase (RNAP). The anti-RT MAbs bind to a peptide comprising residues 294–305 of the RT amino acid sequence. Computer analyses revealed sequence similarity between this peptide and two regions of the RNAP β′ subunit. MAb-binding studies using RT mutants suggested that the epitope is located to amino acids 652–663 of the β′ sequence. One of the MAbs which inhibited the polymerase activity of RT also mediated a dose dependent inhibition of the RNAP activity.

Key words: *Escherichia coli* RNA polymerase; HIV-1 reverse transcriptase; Monoclonal antibody; Cross-reactivity; Sequence comparison

1. Introduction

Escherichia coli RNA polymerase (RNAP) and human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) are polymerases which functionally work in opposite directions. RT catalyzes the synthesis of double-stranded (ds) DNA using the viral single-stranded (ss) RNA as template [1,2] while RNAP uses dsDNA as template for ssRNA synthesis. HIV-1 RT is composed of two subunits with molecular weights of 66 and 51 kDa (p66 and p51), where the amino acid sequence of p51 is identical to the N-terminal sequence of p66 [3–5]. All catalytic activities have been assigned to the p66 subunit of the heterodimer [6–8].

E. coli RNAP contains a catalytic core of two identical α subunits (40 kDa), one β subunit (150 kDa) and one β′ subunit (160 kDa). In vitro the enzyme can utilize dsDNA and ssDNA molecules as template for synthesis of ssRNA and DNA–RNA hybrids, respectively. Part of the catalytic site for polymerization resides in the β subunit of the RNAP [9,10], but also the β′ subunit, the most basic of the RNAP subunits, has been shown to bind DNA in vitro and to be involved in the catalytic activity [11–13]. The sequence of β′ comprises 1407 amino acids and contains eight conserved segments denoted A–H with segment A being characterized by a zinc finger motif [14] and region C by a DNA polymerase motif [15].

The crystal structures of several small polymerases including HIV-1 RT, have revealed a common three dimensional (3D) structure resembling a right hand where finger-, palm- and thumb sub-domains form a cleft which accommodates the substrate [16–21]. A similar active site cleft was visualized by electron crystallography for the *E. coli* RNAP holoenzyme as well as for the RNAP II from yeast [22–24]. Besides their 3D structural similarity, RT and RNAP share another similar feature. Both enzymes bind to ss templates generating a transient DNA–RNA hybrid. In this study, cross-reactivity of four anti-RT monoclonal antibodies (MAbs) with the RNAP β′ subunit is reported. Comparison of the MAb-binding RT sequence with the sequence of β′ revealed a potential common epitope. Inhibition of both the RT and RNAP activity by the same anti-RT MAb further indicated that this epitope is part of a region which is involved in the catalytic activity of the two polymerases.

2. Materials and methods

2.1. MAbs and synthetic peptides

Production and characterization of the anti-RT MAbs as well as the synthetic peptide, Pep 1 (aa (amino acid) 294–318), were previously described [25] (Table 1A). All MAbs recognized both the p66 and p51 subunits of RT in Western blot analysis. The MAb-binding epitopes were mapped to the following RT regions: aa 193–284 (11G10), aa 294–318 (31D6, 31G8, 32E7, 33D5, 5B2) and aa 334–410 (7D8). The synthetic peptides Pep 2 (aa 294–305) and 3 (aa 306–318) were provided by H.C. Holmes, Medical Research Council, London, UK.

2.2. Enzymes used for testing cross-reactivity

The following DNA-binding enzymes were used for testing the MAbs for cross-reactivity in enzyme-linked immunosorbent assay (ELISA): *E. coli* RNAP, T4 RNAP, *E. coli* DNA polymerase, T4 DNA polymerase, *Acinetobacter calcoaceticus* DNA polymerase and DNase, *E. coli* Exo III, *E. coli* Endo III, and an AP endonuclease from MPC cells (all provided by J.R. Lillehaug, University of Bergen); Sp6 RNAP, T7 RNAP, *E. coli* RNaseH and AMV-RT (all from Promega); bovine RNaseA and bovine DNase I (Sigma) and MoMLV-RT (Stratagene). In addition, a Western blot kit for HIV-2 (Pasteur) and an ELISA kit for HTLV-I (Du Pont) were employed for testing with HIV-2 and HTLV-I RT, respectively.

2.3. ELISA and Western blot analysis

In ELISA, at least 200 ng of protein was used for coating of each

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Abbreviations: RT, reverse transcriptase; RNAP, RNA polymerase; MAb, monoclonal antibody; HIV-1, human immunodeficiency virus type 1; ds, double-stranded; ss, single-stranded; aa, amino acid(s); 3D, three dimensional; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction

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microtiter plate well. In Western blot analysis, *E. coli* proteins from crude lysates were separated on 7% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) [26] and electrophoretically transferred onto nitrocellulose membranes (0.45 μm, Schleicher and Schuell) [27]. Binding of anti-RT MAbs was detected as described [25]. *E. coli* (XL-1 Blue) strains harboring the plasmids pMKSe2 and pT7β′ which over-express the RNAP β and β′ subunits, respectively, were provided by K. Severinov, The State University of New Jersey. Growth of bacteria and induction of the *lac* and *lac* UV5 promotor were performed as described [9,28]. Bacteria were disrupted by freezing followed by incubation at 37°C for 1 h in a buffer containing 10 mM sodium phosphate pH 7.2, 8 M urea, 1% SDS and 1% β-mercaptoethanol. No insoluble material was visible after this treatment.

2.4. Computer-assisted analysis

Comparisons of the protein sequences of the HIV-1 Pol polyprotein (consisting of protease, RT and integrase; HXB2 isolate, SWISS-PROT accession no. P04585) and the *E. coli* RNAP β′ subunit (SWISS-PROT accession no. P00577) were performed with the program Compare. Segments in common between the sequences were visualized with the program DotPlot. The program BestFit was used to determine regions in the RNAP β′ subunit sequence with the highest similarity to a defined RT segment. The programs are part of the Wisconsin Sequence Analysis Package, Version 10.0 (Genetics Computer Group, Inc., Madison, WI, USA; URL: http//www.gcg.com/); they were run on a server at the Norwegian EMBnet node (URL: http://www.no.embnet.org/).

2.5. Site-specific mutagenesis

For site-specific mutagenesis of the RT protein, the recombinant circle polymerase chain reaction (PCR) method was applied [29]. The plasmid pRSETART, a derivative of pRSETA (Invitrogen) containing the RT coding region from a HIV-1 HXB2 isolate (EMBL database accession no. K03455) was used as template; circular permuted molecules of pRSETART were generated using the 'outside' primers 5′-TGGTTCCTCTAAGGAGTTTACATAATTGCC and 5′-GAGATTCTAAAAGAACCAGTACATGGAGTG (with their 5′ ends corresponding to HIV-1 HXB2 antisense nucleotide 6312 and sense nucleotide 3471, respectively) and the following mutating primers (mutations underlined) and their reverse complements: 5′-TACCACTAACATTAGAGGCCGAGCTAGAAC (corresponding to HIV-1 HXB2 antisense nucleotides 3427–3456, for mutant Mut 1), 5′-TACCACTAACATTAGAGGCCCAGCTAGAAC (corresponding to HIV-1 HXB2 sense nucleotides 3427–3456, for Mut 2), 5′-AAGAAGCAGAGGCCGAAGTGGCAGAAAACAGAG (corresponding to HIV-1 HXB2 sense nucleotides 3439–3471, for Mut 3 construction). Recombinant DNA circles formed from the permuted molecules via de- and renaturation were transformed into *E. coli* strain JM109. The mutations create an additional *Hae*III restriction site used to select the desired clones. The mutations were verified by DNA sequencing. PCR amplification was performed on a DNA Thermal Cycler (Perkin-Elmer). *Taq* DNA polymerase was purchased from Stratagene. The DNA sequencing kit, plasmid miniprep kit and *Hae*III restriction enzyme were from United States Biochemicals, Qiagen and New England BioLabs, respectively. Oligonucleotides were synthesized on a PCR-MATE 391 DNA synthesizer (Applied Biosystems).

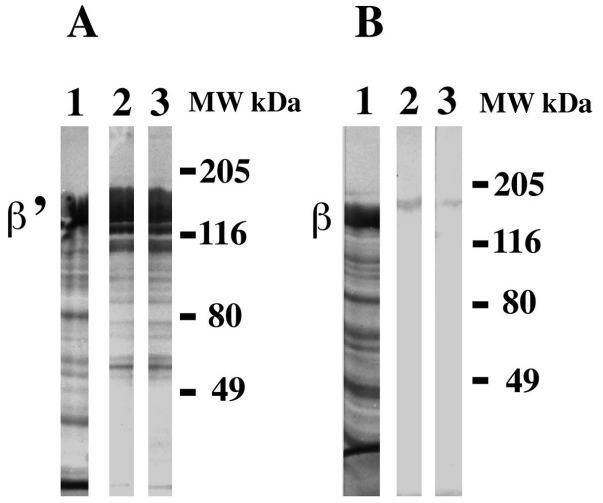

Fig. 1. Western blot analysis of *E. coli* XL-1 Blue strains over-expressing the β′ (A) and β (B) subunits. Lanes 1 (A and B), proteins stained by amido black; lanes 2 (A and B), proteins detected by MAb 31D6; lanes 3 (A and B), proteins detected by MAb 31G8. The molecular weights of pre-stained high molecular weight standard proteins (Bio-Rad) are indicated to the right of lanes 3.

2.6. RNAP assay

The enzyme assay was performed in transcription buffer (Promega) with a final concentration of 25 U/ml RNasin, 0.9 mM dithiothreitol, 0.2 mM of each ATP, GTP and CTP, 0.1 mM unlabelled UTP, 1 μg/ml PhiX174 ssDNA (Bethesda Research Lab.) or PhiX174 dsDNA (Promega), 50 μCi/ml [^{3}H]UTP (NEN, 35.2 Ci/mmol) and 50 μg/ml (7.6 U/ml) RNAP (Promega). Aliquots of 10 μl were removed from the reaction mixture and spotted on DE81 filters (Whatman) after 15, 30, 60 and 120 min reaction time at 37°C. The filters were air-dried, washed with 2×SSC (twice) and ethanol (twice), dried and counted in a liquid scintillation counter. For inhibition of RNAP, the enzyme was pre-incubated with different amounts of anti-RT MAb 31D6 (0.75–6 μg/ml) overnight at 4°C. As a negative control, the same amount of an anti-TrpE MAb was used [25].

3. Results

3.1. Cross-reactivity of anti-RT MAbs

In order to detect similarities between HIV-1 RT and other DNA- and RNA-binding proteins, a panel of 20 anti-RT MAbs recognizing five different epitopes of RT [25] were tested in ELISA using total *E. coli* lysates and the enzymes

Table 1
Reaction patterns of anti-RT MAbs with synthetic peptides and RT mutants

	MAbs (epitopes)				
	11G10 (193–284)	31D6 (294–318)	31G8 32E7 33D5 (294–318)	5B2 (294–318)	7D8 (334–410)
A: *RT peptides*					
Pep 1: aa 294–318 PLTEEAELELAENREILKEPVHGVY	−	+	+	+	−
Pep 2: aa 294–305 PLTEEELELAE	−	+	+	−	−
Pep 3: aa 306–318 NREILKEPVHGVY	−	−	−	+	−
B: *RT mutants*					
Mut 1: 294-PLT**L**EAELELAE-305	+	−	ND	+	+
Mut 2: 294-PLTLEA**Q**LELAE-305	+	−	ND	−	−
Mut 3: 294-PLTEEAEA**E**VAE-305	+	+	ND	+	+

ELISA was used to detect MAb-binding to the peptides (A). Western blot was used to detect binding to the mutants (B). Amino acid residues are written with their single-letter abbreviation, mutations are in bold. +/− denotes binding/no binding. ND, not tested.

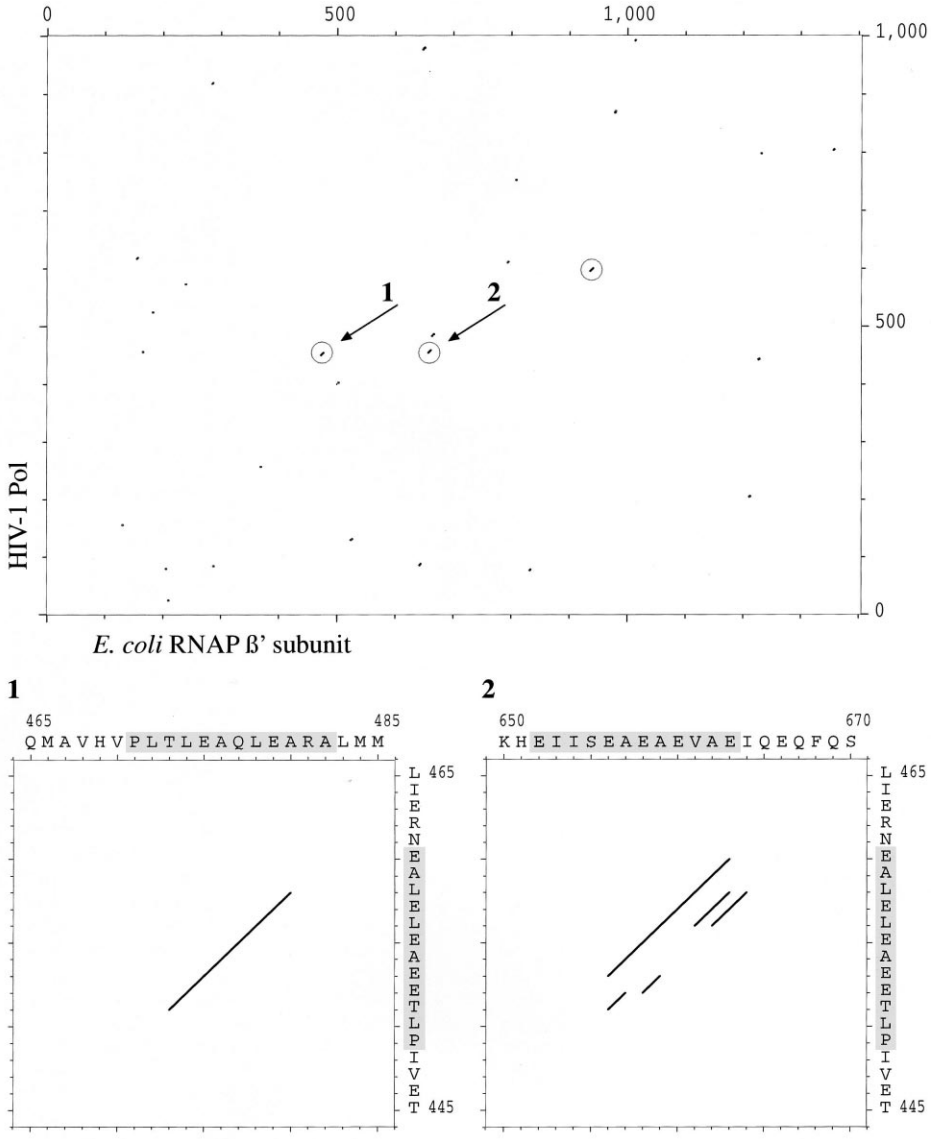

Fig. 2. Dot-plots visualizing the similarity between the HIV-1 RT and *E. coli* RNAP β′ subunit amino acid sequences. The HIV-1 Pol polyprotein sequence (vertical axis; RT from position 156 to 721) and the *E. coli* RNAP β′ subunit sequence (horizontal axis) were compared in any possible register using a window size of 12. Dots in the upper plot represent window scores meeting a high match criterion (a stringency of 25) above which almost all scores are filtered out; dots in the two lower plots (enlargement of the relevant segments) represent window scores greater than or equal to 8. Dots are positioned in the middle of the window on both axes. The scores are determined by summing the match values for each pair of residues within the window at each window position using the default PAM-250 matrix (see program documentation). Amino acid residues along the axes of the lower plots are written with their single-letter abbreviations. Residues on shaded background comprise the potential RT and RNAP β′ subunit epitopes.

listed in Section 2.2 as antigens. Cross-reaction was observed for anti-RT MAbs 31D6, 31G8, 32E7 and 33D5 with *E. coli* RNAP, T4 RNAP and *E. coli* lysates. T4 RNAP contains the *E. coli* host RNAP α, β and β′ subunits. The results therefore indicated a specific binding of the MAbs to the *E. coli* RNAP core enzyme. The particular RNAP subunit recognized by the MAbs was determined by Western blot analysis using bacterial strains over-expressing either the β or β′ subunit of RNAP (Fig. 1). The molecular weight and relative amounts of the β′ and β subunits are shown by amido black staining in lanes 1 (Fig. 1A,B). Lanes 2 and 3 (Fig. 1A,B) demonstrate binding of the MAbs (31D6 and 31G8, respectively) to the β′ subunit. Binding generated solid bands with the β′ over-expressing strain (Fig. 1A) and can even be detected with the β over-expressing strain as thin bands visible above the position of the β subunit. Such thin bands demonstrating cross-reactivity of the MAbs with the β′ subunit were also observed by Western blot analysis of the *E. coli* strain HB101 (not shown).

3.2. RT peptides recognized by the anti-RT MAbs

It was previously shown that the MAbs 31D6, 31G8, 32E7, 33D5 and 5B2 bind to a synthetic peptide comprising aa 294–318 of the HIV-1 RT sequence [25] (Pep 1; Table 1A). In this study, two synthetic peptides consisting of the N- and C-terminal halves of Pep 1 (Pep 2, aa 294–305, and Pep 3, aa 306–318, respectively) were used in order to determine the sequence recognized by the MAbs more precisely and, furthermore, to differentiate between the MAbs. Binding to peptide

Pep 2 was demonstrated for the MAbs 31D6, 31G8, 32E7 and 33D5 and binding to peptide Pep 3 for the MAb 5B2 (Table 1A). In contrast to the first four MAbs, MAb 5B2 did not recognize the RNAP β′ subunit.

3.3. Sequence comparison of RT and the RNAP β′ subunit

In order to determine whether the cross-reactivity of the anti-RT MAbs with RNAP β′ is reflected at the primary sequence level of the two proteins, a computer-assisted sequence comparison was performed using the programs Compare and DotPlot. Similar segments in the two sequences according to highly stringent match criteria are indicated by dots in the upper dot-plot of Fig. 2. Interestingly, of the three most pronounced signals remaining under these conditions (circled), two are from pairs of matching sequence segments both involving the RT peptide sequence recognized by the cross-reacting MAbs (arrows; see enlargements in the lower dot-plots). The RNAP β′ subunit partner segments in the pairs are the sequences 471-**PLTLEAQLE**ARA-482 and 652-E**II-SEAEAEVAE**-663, with residues having identical or similar counterparts in the RT Pep 2 sequence written in bold. Support for the significance of this finding was provided by another comparison procedure, the program BestFit, which reveals the optimal alignment of the best segment of similarity between two sequences. When running the program repeatedly with sequence pairs containing the RT Pep 2 sequence and overlapping segments of the RNAP β′ subunit sequence 250 amino acids in length, the best RT Pep 2 sequence partner found within RNAP β′ according to quality-score calculation by the program is a segment included in the aa 471–482 portion, succeeded by a segment included in the aa 652–663 portion (data not shown).

3.4. Binding of the MAbs to RT mutants

Mutations were introduced into the RT sequence in order to determine which of the β′ amino acid residues were recognized by the cross-reacting anti-RT MAbs (Table 1B). The mutations rendered the RT sequence aa 294–305 more similar to the RNAP β′ sequences 471-PLTLEAQLEARA-482 (Mut 1 and Mut 2) and 652-EIISEAEAEVAE-663 (Mut 3) found as best hits in sequence comparison. Binding of MAb 31D6 was abolished by the mutations in Mut 1 and 2. MAb 11G10 binding to an epitope located N-terminal to the mutated sequence recognized both mutants whereas MAbs 5B2 and 7D8 binding to epitopes located C-terminal recognized Mut 1 but not Mut 2. This indicated that the second of these mutations introduced conformational changes also in the epitopes of these MAbs. Mutant Mut 3, in contrast, was recognized by MAb 31D6 which strongly suggested that residues within the β′ sequence 652-EIISEAEAEVAE-663 accounted for binding of MAb 31D6 to the β′ subunit. Furthermore, binding also of the other MAbs indicated that the folding of the RT epitopes recognized by them was not affected by the mutations in Mut 3.

3.5. Inhibition of RNAP by anti-RT MAb 31D6

MAb 31D6 was previously shown to inhibit the polymerase activity of RT while no inhibition of the RNaseH activity was observed. This indicates that inhibition of the RT polymerase activity was not a result of any unspecific effect [25]. RNAP assays were performed in the presence of MAb 31D6 or a non-specific anti-TrpE MAb in order to determine if the regions in RNAP β′ responsible for the cross-reactivity of the MAbs mediate a functional similarity in the two enzymes. RNAP was incubated with the MAbs prior to addition of the substrates. Using ss PhiX DNA as template, inhibition was demonstrated both by kinetic analysis (Fig. 3A) and by a dose–response assay (Fig. 3B). A significant inhibition of RNAP activity by MAb 31D6 was also observed by kinetic analysis using the ds PhiX DNA as template (not shown).

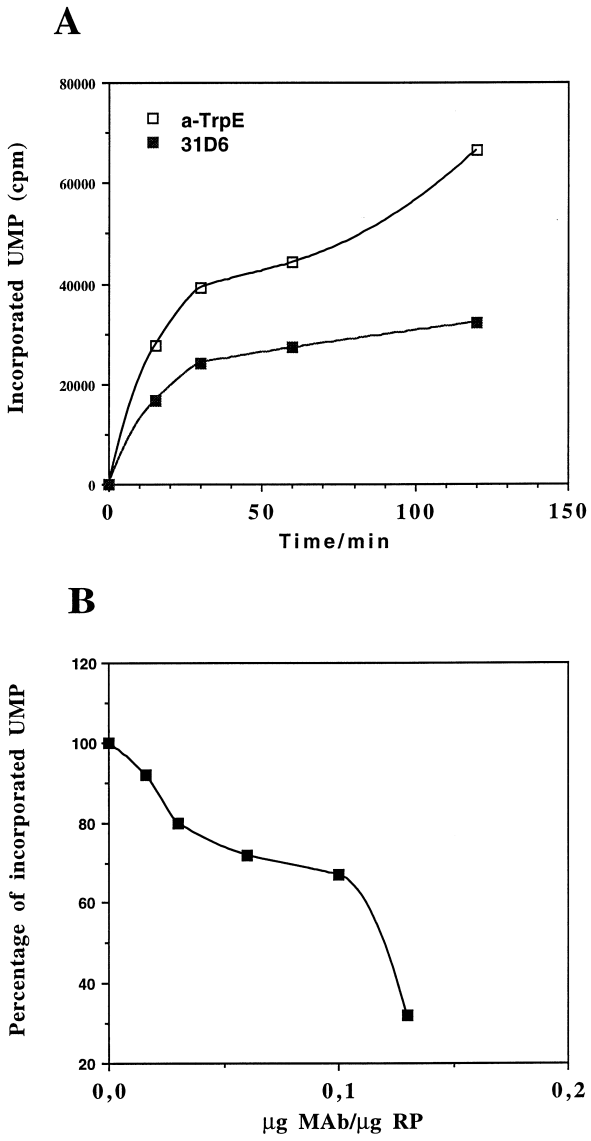

Fig. 3. Inhibition of RNAP activities by MAb 31D6. A: Kinetics of in vitro RNAP activity with ssDNA template in the presence of MAb 31D6 or an anti-TrpE MAb. Before being tested for activity, 2 μg RNAP was incubated overnight at 4°C with the 0.4 μg MAbs. B: Dose–response dependent inhibition of RNAP activity with ssDNA template in the presence of MAb 31D6. The activity of RNAP was defined as 100% activity after pre-incubation with the same amount of anti-TrpE MAb.

4. Discussion

Computer-assisted search for similarities between protein sequences is based upon comparing chemical and biological properties of their amino acid residues. For the described

polymerases, this has been insufficient to prove the evolutionary relationship between them [21]. Antibodies, on the other hand, recognize 3D structures on the surface of antigens and therefore can be considered as complementary tools for protein relationship analysis. Amino acid residues of the hypervariable domains of the antibody molecule interact with residues of the antigenic epitope. Still, mapping of epitopes using synthetic peptides is usually unsuccessful. This may be because the folding of peptides does not mimic the conformation of the epitope or because the number of amino acid residues in the peptide is insufficient for binding. The cross-reacting anti-RT MAbs presented in this study, however, recognized a synthetic peptide consisting of the RT amino acid residues 294-PLTEEAELELAE-305. According to the 3D model of the RT structure, this sequence is located on α-helix J in the thumb sub-domain of both the p66 and p51 subunits. In p66, the thumb sub-domain is involved in the formation of the polymerase cleft. In the p51 subunit, on the other hand, α-helix J participates in dimer formation and is not part of the structural elements that make potential contacts with DNA [16–18]. It cannot be concluded from the present study which of the subunits or whether both of them are subjected to inhibition by MAb 31D6.

Cross-reactivity of the anti-RT MAbs with the β′ subunit of *E. coli* RNAP suggests a structural similarity of the two enzymes. Having defined the specific RT sequence recognized by the MAbs made it possible to search for sequence similarity in the sequence of RNAP β′. Computer-assisted analysis revealed the β′ sequences 471-PLTLEAQLEARA-482 and 652-EIISEAEAEVAE-663 as the first and second best best matching hits. Site-directed mutagenesis of the RT sequence then demonstrated that the second β′ sequence (652–663) was recognized by the anti-RT MAb 31D6. This sequence is located in the conserved region E of the β′ subunit [14]. Interestingly, another study based on a complementary assay using an *E. coli* strain unable to synthesize the β′ chain at 37°C showed that the mutation of a residue within this sequence (E656) rendered the enzyme recessive negative [11]. Inhibition of RNAP by the anti-RT MAb 31D6 confirms the functional importance of this β′ region. Furthermore, inhibition of both HIV-1 RT and *E. coli* RNAP by the same anti-RT MAb suggests common functional properties of the two enzymes. From the results of this study, it is likely that they are at least partly determined by the sequences recognized by MAb 31D6, i.e. the sequence 294-PLTEEAELELAE-305 in HIV-1 RT and 652-EIISEAEAEVAE-663 in the *E. coli* RNAP β′ subunit.

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