nucleotides (in contrast to trimers and trinucleotide diposphates, which could be subjected to the method\(^3\); this limitation is based on the requirement of terminal transferase for a minimum primer chain lengths of three nucleotides. As to the upper chain lengths, undercaturated nucleotides of specific sequences\(^4\) and the homopolydides poly(dC-T-G), poly(dA-A-T) and poly- 
\(d(A-T-G)\)\(^5\) have been tested successfully so far. It seems therefore likely that also polynucletides with specific sequences of higher chain length can be labeled by the method described here, as long as contaminating nucleases are excluded from the reactions.

Oligodexoxynucleotides carrying a 3'-phosphomonoester group are not accepted as primers for terminal transferase; such limitation, however, may be easily circumvented by phosphatase treatment of the oligonucleotide to be labeled, before the terminal addition reaction is carried out. In this case care must be taken not to carry phosphatase into the terminal addition reaction, as ribonuclease triphosphates are readily degraded by phosphatase.

In special circumstances (for instance for studies with 3'-specific phosphatase), it may be necessary to have oligodeoxynucleotides available with \(^32\)P-labeled phosphomonoester groups at the 3'-ends. For this purpose, elimination of the ribonuclease residues is possible by periodate and amine treatment\(^6\); by this reaction for instance \((A-C-C-A)\) \(^32\)P\(\alpha\) is converted to \((A-C-C-A)\) \(^32\)P\(\alpha\).

Partial Sequence Analysis. One crucial point of this method consists in meeting the proper conditions for the partial digestion of the oligonucleotide to be analyzed with snake venom phosphodiesterase. In fact, unsuccessful experiments led to be encountered a few times owing to insufficient digestion or to over digestion of the oligonucleotide material. Since the priming activity necessary for the terminal addition reaction decreases with the chain length of the primer,\(^7\) the ideal digestion condition would be those in which more nucleases of smaller oligonucleotides are produced with very few undigested molecules remaining. Thus, the extent of digestion has to be balanced carefully by variation of enzyme concentration or incubation time.

Partial sequence determination on semimicroscale is possible with as little as 0.8 \(\mu\)g units of the synthetic octamernucleotide \((A-C-G-A-T-C-C-A)\), whereby only the two nucleotides toward the 3'-end remain unidentified (Fig. 3).

Partial sequence determination on microscale can be carried out with as little as \(5 \times 10^{-10}\) \(\mu\)g units of the same oligonucleotide (Fig. 5); in this case, however, three nucleotides from the 5'-end remain unidentified. It should be pointed out that the fingerprint analyses of labeled oligomer- 
ineotide mixtures allow additional information in respect to mononucleotide residues sequentially released by snake venom phosphodiesterase, thus, according to the fingerprint rules\(^8\) as the relative position of spot IV to V of Fig. 5 evidences the removal of one thymidylic acid residue positioned at the fourth place counted from the 3'-end of the original oligonucleotide; likewise the relative positions of spots II to III and II to IV indicate the removal of cytidylic acid residues. This information confirms or even supple- 
ments the results obtained from the identification of the 2-terminal nucleotides of the individual labeled oligonucleotides by spleen phospho- 
diesterase degradation. In contrast to this, partial sequence determination on semimicroscale is based exclusively on the identification of the 3'-ter- 
ninal nucleotides by spleen phosphodiesterase degradation, since separation of the labeled oligonucleotides in this case is carried out by DEAE-cellulose column chromatography (Fig. 3), not by the fingerprint technique.

Acknowledgment

This work was supported by grants from the Deutsche Forschungsgemeinschaft, which we are gratefully acknowledged. H. Fuss wishes to thank O. v. Fugger, R. Grimmel, and V. Long for introduction into the two-dimensional fingerprint technique. Valuable technical assistance is credited to Mau S. Sharp.

[27] A New Approach to the Study of Nucleotide Sequences in DNA: The Analysis of Termini Formed by DNases

By Giorgio Bernardi, Stanislav D. Emrich, and Jean-Paul Thiery

We describe here a method developed in our laboratory for characterizing and comparing nucleotide sequences in DNA's. The method is based upon the fact that DNA's are sequence-specific in that is, they are able to recognize the nucleotides near the phosphodiester bonds which they split. We have demonstrated this point for at least the three DNA's we have investigated so far: spleen acid DNA, small acid DNA, and pancreatic DNA. The spleen enzyme, for instance, is able to recognize at least the two nucleotide pairs lying on one side of the breaks; we call termini the terminal and penultimate nucleotides, W X Y Z (Fig. 1).

Since DNA's are sequence-specific, the analysis of termini, namely, the determination of the base composition of the termini released from the sequences split by DNA's, provides information on the frequency of these sequences in the DNA's analyzed. The analysis of termini is characterized
Fig. 1. A scheme of degradation of DNA by a DNase. The phosphate is arbitrarily put at the newly formed 3' end.

by two features, which distinguish it from a determination of the frequency of the sequences split by the enzymes (Fig. 2): (a) the analysis of termini provides the composition of each terminus as derived from all the split sequences; (b) since different nucleotide sequences can be split with different $k_n$ and $V_m$ values, the analysis of termini is related to an apparent frequency, not to the real frequency, of the split sequences.

It can be expected, and it is experimentally found, that (a) the composition of each terminus released from bacterial DNA's having different basic compositions is linearly related to their G + C contents; and (b) the composition of termini released from DNA's containing "repetitive"

Fig. 2. A scheme of the analysis of termini: (a) Sequences 1 to 5 are recognized and split by the enzymes. (b) Ends are formed from sequences 1 to 5 proportionately to constants $K_1$ to $K_5$, which depend upon the $k_n$ and $V_m$ values for sequences 1 to 5. Termini deriving from each position of the terminal doublet of all sequence split are isolated, and their composition is determined.

nucleotide sequences deviates, by excess or by defect, from that expected for "nonsuppressive" DNA's having the same G + C contents. The deviation plots which can be thus obtained represent a novel way of characterizing "repetitive" DNA's.

Materials and General Procedures

DNA samples to be submitted to the analysis of termini were routinely purified by chromatography on hydroxyapatite columns from contaminating RNA, oligonucleotides, and ultraviolet-absorbing materials.1 Enzymes. Spleen DNase B, spleen phosphodiesterase B, spleen exonuclease, and snake venom exonuclease were preparations obtained according to described procedures.2,3 Small DNase4 was a preparation obtained following a method to be published. Pancreatic DNase was a commercial preparation (code D; Worthington, Freehold, New Jersey). For the definition of enzyme units see references cited in footnotes.4,5,6

DNase digestions were routinely performed at room temperature on native DNA ($A_{600}=8$ or 0.8). In the case of spleen and small DNases, DNA solutions in 50 mM ammonium acetate-1 mM EDTA, pH 5.5 were used; enzyme concentrations in the incubation mixture were 0.1-1 unit/ml. In the case of pancreatic DNase, digestions were carried out in 50 mM Tris-HCl-10 mM MgCl2 (or MnCl2), pH 7.6, with 0.02-0.2 μg of enzyme per milliliter of incubation mixture. The use of different DNases implying different digestion conditions as far as pH, ionic strength, and metal ions are concerned, the resulting oligonucleotides were dialyzed against running distilled water (see below) before further treatments.

The termini of oligonucleotides released by the small and pancreatic DNase do not vary with the digestion level; some variation takes place during digestion by the spleen DNase, an enzyme which hydrolyzes DNA according to two different mechanisms (diploenzyme and haploenzyme). In this latter case, which was investigated in detail,7,8 the termini released from

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different DNA's were always compared at identical oligonucleotide size levels. A convenient way to prepare the oligonucleotides having the desired average chain length ($P_\text{av}$) is to use the relationship existing between the hypochromic shift (HS) undergone by DNA and the reciprocal average size ($P_\text{av}^{-1}$) of the oligonucleotides. For the spleen and small DNAs a linear relationship is obtained; using the digestion conditions described above, at 22°C,

$$P_\text{av}^{-1} = 0.0086 \cdot \text{HS} \cdot (G + C) + 0.01$$

where HS is expressed as percentage value and $G + C$ as molar fraction; this equation is valid for $P_\text{av}^{-1}$ values comprised between 0.02 and 0.1.

For pancreatic DNase the equivalent equation determined on calf thymus DNA is

$$P_\text{av}^{-1} = 0.0028 \cdot \text{HS} + 0.025$$

which is valid for $P_\text{av}^{-1}$ values comprised between 0.035 and 0.1.

Diagnosis of Oligonucleotides. Vials containing $200 \mu$g of the oligonucleotides were boiled successively in 10% sodium carbonate 0.1 M EDTA, 0.05 M ammonium acetate buffer, pH 5.5, and water. Dialysis was done at 4°C against water flowing through a glass tube having a diameter only slightly larger than the dialysis tubing. Several oligonucleotide samples (2 $A_{260}$ units at a concentration of 1-10 $A_{260}$ units/ml) were dialyzed simultaneously against 10-25 l of distilled water for 8-14 hours. Under these conditions, even dinucleotides, when present in less than 2-3% amount, were quantitatively retained.

DEAE-cellulose (Sera, Heidelberg, Germany; 0.57 meq/g) was washed in succession with 0.1 N NaOH, water, 0.1 N HCl, water, and 2 M ammonium acetate, pH 5.5. Only the finest particles, obtained by decantation and forming 1-2% of the material, were used. The column was packed from a suspension of DEAE-cellulose in 2 M ammonium acetate (about 1-L, v/v), and was washed with 2 M ammonium acetate and water immediately before use.

Average chain length or average degree of polymerization, $P_\text{av}$, of the oligonucleotides obtained by DNase digestion was determined as described in the following section.

Nucleotide analyses were done on Bio-Gel P-2 columns, or Sephadex G-10 (Pharmacia, Uppsala, Sweden) columns, or, more recently, by the following modifications of the method of Usel et al. of 0.02 $A_{260}$ unit of


nucleotides in 5 ml of 0.4 ammonium formate, pH 4.7, were loaded on a 0.2 x 5 cm column of Antron A6 (Bio-Rad, Richmond, California), equilibrated with the same solvent; elution was done at room temperature using a flow rate of 0.75 ml per hour; a typical separation required about 90 minutes; the detection and evaluation of the nucleotide peaks were done as already described.

Methods for the Determination of Terminal and Penultimate Nucleotides

**Determination of 3' Terminal Nucleotides**

Figure 3 shows the principle of the method as applied to 3'-P oligonucleotides. Oligonucleotides are first dephosphorylated, then hydrolyzed by spleen exonuclease, an enzyme which degrades oligonucleotides, starting from their 5' terminal ends and releasing 3'-mononucleotides. The 3' terminals, liberated as nucleosides, are separated from nucleotides, and analyzed. The same procedure can be applied to 5'-P oligonucleotides; in this case, dephosphorylation has the purpose of allowing spleen exonuclease to act. A typical determination is performed with 0.1 unit/ml (final concentration)

![Diagram of nucleotide extraction](attachment:3.png)

**Dephosphorylation.** Ammonium acetate 1 M EDTA 20 mM, pH 5.5, is added to dialyzed oligonucleotides to reach 10-20 mM concentration of acetate. Digestion is performed with 0.1 unit/ml (final concentration)
of spleen acid phosphomonoesterase at room temperature for 14 hours. The enzyme is inactivated by shaking the solution vigorously for 5 minutes with 1/4 A<sub>380</sub> to 1/2 volume of a chlorform-isooamyl alcohol (CA) (24:1, v/v) mixture.

Eomucosal Digestion. This is done in a quartz cuvette in order to follow continuously the A<sub>380</sub> increase; 0.2 unit of spleen eosinoclease is added per A<sub>380</sub> unit of oligonucleotides. Digestion takes about 1 hour at room temperature; the end of reaction is indicated by a plateau of hyperchromicity. In the case of oligonucleotides having an average chain length higher than 15, 0.2 unit of spleen acid DNase is added; this is necessary to assure the complete degradation of oligonucleotides which are resistant to eosinoclease.

Separation of 5′ terminal nucleotides is accomplished on either a DEAE-cellulose or a QAE A-25 Sephadex column. Spleen eosinoclease digests are loaded on 0.5 X 10 cm DEAE-cellulose column previously washed with 10 ml of 2 M ammonium acetate, pH 5.5, and then with 10 ml of water using a 20 ml per hour flow rate. UV-absorbance of the column effluent is monitored. Nucleotides are washed out with 3-5 ml of water using a flow rate of 0 ml per hour; nucleotides are eluted by a step of 1 M ammonium acetate, pH 5.5, at a 20 ml per hour flow rate. Alternatively, the separation can be accomplished on 0.4 X 7 cm QAE Sephadex column, washed before use for 2 hours with 2 M NH₄Ac-7 M urea and then for 3 hours with water at a 4 ml per hour flow rate. QAE-Sepabeads is more suitable for work with oligonucleotides of average chain length greater than 50, since its higher capacity permits loading of greater amounts of eosinoclease digests.

The average oligonucleotide chain length, P, is taken to be equal to the ratio (A<sub>380</sub> + A<sub>280</sub>)/A<sub>350</sub> where N<sub>s</sub> and N<sub>e</sub> indicate nucleotides and (terminal) nucleotides, respectively.

Nucleotide analysis of 5′ terminals is accomplished as described in the previous section.

Determination of 5′ Terminal and 5′ Penultimate Nucleotides<sup>2</sup>

The procedure is shown in Fig. 4. Oligonucleotides are dephosphorylated, treated with pancreatic DNase in order to decrease their average size, and digested with venom exonuclease. This enzyme degrades oligonucleotides starting from the 3′ end and splits off one 5′ nucleotide at a time. 5′ Terminal dinucleotide monophosphates, being very resistant to digestion, accumulate in the digestion mixture and can be isolated by DEAE-cellulose chromatography. They are then split with spleen exonuclease to 3′ nucleotides (corresponding to the 5′ terminals of oligo-nucleotides) and nucleotides (5′ penultimates of oligonucleotides). These are subsequently separated and analyzed. A typical experiment is described. Digests and dephosphorylation are done as described above except that

6 A<sub>380</sub> units of oligo-nucleotides in 0.8 ml of buffer are treated and that the phosphatase digestion is done in 1 mM Tris-acetate, pH 5-5.5.

Pancreatic DNase Treatment. Dialyzed, dephosphorylated oligonucleotides, 0.2 ml, are lyophilized and dissolved in 0.25 ml of 5 mM MgCl₂. The pH is adjusted to 7.3-8 with 1 M Tris; 2 μl of pancreatic DNase solution (0.2 mg/ml in 10 mM MgCl₂) are added, and the sample is incubated at 37°C for 10 minutes.

Venom Eosinoclease Digestion. The pH of pancreatic DNase digest is adjusted to 8.8-9.0 with 1 M Tris. The sample is transferred to a quartz cuvette (0.1 cm optical path) and incubated at 37°C with 20 μl of venom exonuclease (2 units/ml). The increase of A<sub>380</sub> is followed; it is rapid in the first phase of digestion, then becomes progressively slower. When the slow phase of A<sub>380</sub> increase has been reached, the yield of dinucleotide monophosphates is close to 50%; if digestion is continued, the yield decreases as some hydrolysis of dinucleoside monophosphates takes place. The reaction is stopped by diluting the sample with 0.5 ml of 4 mM acetic acid and shaking vigorously for 5 minutes with 0.2 ml of CA.

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**Fig. 4.** A scheme of determination of the 5′ terminal and 5′ penultimate nucleotide compositions.
Isolation of Dinucleoside Monophosphates. Nucleosome digests are loaded on 0.5 x 10 cm DEAE-cellulose columns, washed previously with 1 M ammonium acetate for 1 hour and then with water for 2 hours at a flow rate of 6 ml per hour. The ultraviolet absorbance of column effluent is monitored. Nucleosides are eluted with water and then a linear gradient of ammonium acetate, pH 7.6, is started (0-1.5 M, total volume 100 ml). As soon as dinucleoside monophosphates are eluted, the slope of the gradient is increased by reducing the total gradient volume to 15 ml. To achieve the separation, it is essential to use the finest particles, forming less than 5% of DEAE-cellulose. These can be prepared by sieving or by decantation.

Sphero-Eluotrace Digestion. Dinucleoside monophosphates are lyophilized after chromatography on DEAE-cellulose, dissolved in 0.6 ml of 17 mM ammonium acetate and digested at 22° with 10 ml of spleen exonuclease (40 units/ml). The ammonium increase is followed; its maximum indicates the end of the reaction (about 30 minutes).

DEAE-cellulose chromatography of spleen exonuclease digest is done on 0.5 x 7 cm DEAE-cellulose columns, washed previously for 1 hour with 1 M ammonium acetate and then for 1.5 hours with water using a flow rate of 6 ml/hour. Nucleosides (5’ penultimate) are washed out with water, nucleotides (5’ terminals) are subsequently eluted with a step of 0.4 M ammonium acetate.

Dephosphorylation of Nucleotides. Nucleotides are lyophilized in order to get rid of excess of salt and dissolved in 15 mM ammonium acetate, pH 5.5, 1 ml of 0.5% borate serum albumin solution, in water and 0.5% unit of acid phosphomonoesterase are added. Dephosphorylation is carried out for 12-18 hours at 22°. Nucleotides are isolated from the digestion mixture by a DEAE-cellulose column chromatography performed as described in preceding paragraph.

Nucleotide analysis is accomplished as described in the preceding section.

Determination of 3’ Penultimate Nucleotide*

The isolation and analysis of this terminus was performed only on spleen acid DNase digests of calf thymus DNA. We will briefly mention here the two methods used to determine it. The first one (Fig. 9), which can be used for the procedure of dinucleotides, was to split the spleen DNase digest with pancreatic DNase in the presence of Mn²⁺, the dinucleoside triphosphates, which represented about half of the products originated from

Table I: Terminus Released from Different DNAs by Spleen Acid DNase

<table>
<thead>
<tr>
<th>DNA</th>
<th>Nucleotide</th>
<th>3' Terminal</th>
<th>5' Terminal</th>
<th>5' Penultimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilus</td>
<td>G</td>
<td>37</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>22</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>43</td>
<td>22</td>
<td>49</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>G</td>
<td>47</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>17</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>11</td>
<td>39</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>25</td>
<td>17</td>
<td>45</td>
</tr>
<tr>
<td>Micrococcus lactus</td>
<td>G</td>
<td>56</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>12</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>17</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>15</td>
<td>7</td>
<td>32</td>
</tr>
</tbody>
</table>


Composition. The possibility exists, however, that some of the differences merely reflect the composition of the nearest neighbor of the termini actually recognized by the enzymes. Such a possibility can be checked by comparing the experimental results with those expected from the nearest-neighbor data. Figure 6 shows that the composition of the 5' terminal and penultimate nucleotides obtained by spleen DNase digestion is quite different from that expected from nearest-neighbor data; in contrast, in the case of the small enzyme, this is true only for the 5' terminal nucleotide, whereas the composition of the 5' penultimate nucleotide is practically identical with that expected from nearest-neighbor data. Since the 3' terminal nucleotides have a composition different from that expected for the nearest neighbors of the 5' terminal nucleotides (Table II), it should be concluded that the spleen DNase recognizes sequences of at least three nucleotides and the small DNase sequences of at least two nucleotides. In fact, the analysis of the 3' penultimate nucleotide released by the spleen

Table II: Terminus Released from Different DNAs by Spleen Acid DNase

<table>
<thead>
<tr>
<th>DNA</th>
<th>Nucleotide</th>
<th>3' Terminal</th>
<th>5' Terminal</th>
<th>5' Penultimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilus</td>
<td>T</td>
<td>15</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>74</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>G</td>
<td>16</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>66</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>Micrococcus lactus</td>
<td>T</td>
<td>14</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>26</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>56</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4</td>
<td>2</td>
<td>23</td>
</tr>
</tbody>
</table>


Fig. 6. The observed compositions of the 5' terminal and 5' penultimate nucleotides released from bacterial DNAs by the spleen and small DNases are plotted against the compositions calculated for the nearest neighbors of the 3' and 5' terminal nucleotides, respectively. The points are aligned on the broken lines, suggesting a selection. The slopes of lines through the points are shown in the left-hand upper corner of each frame. From J. P. Thiery, S. D. Ehlich, A. Devillers-Thiery, and G. Bernardi, *Eur. J. Biochem.*, submitted for publication; and S. D. Ehlich, A. Devillers-Thiery, and G. Bernardi, *Eur. J. Biochem.*, submitted for publication.
### TABLE III

<table>
<thead>
<tr>
<th></th>
<th>3'-P terminal</th>
<th>3'-P penultimate</th>
<th>5'-OH terminal</th>
<th>5'-OH penultimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>22</td>
<td>(31)</td>
<td>22</td>
<td>(32)</td>
</tr>
<tr>
<td>G</td>
<td>16</td>
<td>(21)</td>
<td>43</td>
<td>(21)</td>
</tr>
<tr>
<td>A</td>
<td>16</td>
<td>(19)</td>
<td>9</td>
<td>(17)</td>
</tr>
</tbody>
</table>


Values in parentheses indicate the composition of each terminus as calculated from the nearest neighbor(s).

DNase from calf thymus DNA strongly indicates that this nucleotide is also recognized by the enzyme (Table III). Finally, it should be mentioned that recent experiments have shown that pancreatic DNase recognizes a sequence of at least three nucleotides.10

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**Fig. 7.** Plot of the percentage of A(T), G(C), C(U), and T(A) in the 3' terminal, 5' terminal, and 3' penultimate nucleotides formed by the spleen and the small DNase from bacterial DNA's as a function of their G + C content. From J. P. Thiery, S. D. Erlich, A. Delville-Thierry, and G. Bernardi, *Eur. J. Biochem.*, submitted for publication; and S. D. Erlich, A. Delville-Thierry, and G. Bernardi, *Eur. J. Biochem.*, submitted for publication.

**Fig. 8.** Differences between the percentages of 3' terminal, 5' terminal, and 3' penultimate nucleotides formed from "parental" DNA's and the corresponding values for bacterial DNA's. From J. P. Thiery, S. D. Erlich, A. Delville-Thierry, and G. Bernardi, *Eur. J. Biochem.*, to be submitted for publication.

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**References:**


It should be pointed out that the sequences which are split are seen only through the termini they release, and that a great part of the possible differences in $K_a$ and $V_{max}$ values associated with individual sequences are lost through the averaging of the compositions of the termini released. In the only case where the sequences which were split could be estimated, the case of poly(OAT-ATAT), A was found to form 90% and 87% of the 4' termini released by the spleen and the small enzyme, respectively, indicating that both enzymes have different $K_a$ and $V_{max}$ for the two equally abundant sequences ATAT and TATA. This finding stresses the fact that the frequencies of the termini as determined by our analysis are only apparent frequencies and should be clearly distinguished from the real frequencies of the termini recognized by the enzymes.

The empirical relationships of Fig. 7 do not hold for DNA's having different distributions of the frequencies of nucleotide sequences split by the enzymes compared to bacterial DNA's, as shown by the deviation plots (Figs. 8 and B) of satellite DNA's from mouse and guinea pig, mitochondrial DNA from yeast, and the DNA's from eukaryotes.5,6

In conclusion, the analysis of termini formed by DNase is a new method for characterizing and comparing nucleotide sequences in DNA's, the deviation plots of Figs. 8 and 9 being a novel approach to the study of "repetitive" nucleotide sequences. It should be noted that, when applied to the 3' terminal, the 5' terminal, and the 5-triphosphate nucleotide, the method, as described here, requires 100 μg of DNA. Radioactive labeling of the 3' terminus with [α-32P]ATP, using the terminal deoxyribonucleotidyl transferase, and the 5' ends with polyethylene glycol (work in progress) should lead to a considerable reduction in costs.


[28] Analysis of Labelled 3' Terminal Nucleotides of DNA Fragments

By UMBERTO BERTAZZONI, STANISLAV D. EHRICH, and GIUSEPPE BERNARDI

We describe here a procedure for the analysis of 3' terminal nucleotides of DNA fragments. The procedure is based on (a) the labeling of 3' ends of oligo- or polynucleotides by the addition of 2 residues of [α-32P]AMP catalyzed by terminal deoxyribonucleotidyl transferase using [α-32P]ATP as a donor; (b) the separation of terminally labeled DNA fragments from excess ATP; (c) the digestion of the fragments with spleen and DNase and exonuclease; (d) the separation of the four labeled terminal nucleotides on DEAE-cellulose columns, under conditions permitting the simultaneous separation of labeled-AMP.

Figure 1 summarizes the two enzymatic steps involved in the procedure.

Materials and Methods

3'-Hyphal oligonucleotides were prepared by degradation of calf thymus DNA by spleen and DNase followed by dephosphorylation of denatured DNA fragments. Their average chain length (average degree of polymerization, $P_a$) and the composition of 3'-terminal nucleotide were determined

1 Erurom science, Ltd. This publication is contribution No. 886 of Eururom Biology Division.