Chapter IV

DNA Polymerases in isolated cell enriched fractions from the rat brain at different ages
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DNA POLYMERASES IN ISOLATED CELL ENRICHED FRACTIONS FROM THE RAT BRAIN AT DIFFERENT AGES

INTRODUCTION:

Previous studies from this laboratory have shown that the DNA content of both rat (Subba Rao and Subba Rao, 1982) and chick brains (Shrivastaw and Subba Rao, 1975) goes up significantly in the later stages of lifespan and this was attributed to one or more of the following possibilities a) replication of glial cells b) an increase in the intracellular DNA not necessarily connected with the replication process but including the DNA repair. Further, when the activity of DNA polymerase (mostly DNA polymerase 3) was examined, it showed a corresponding peak at stages when increased DNA content was observed (Subba Rao and Subba Rao, 1984). This pattern of changes led us to believe that brain probably retains good repair capacity throughout the lifespan.

We have extended these studies to isolated cell enriched fractions. It is generally accepted that brain consists of three distinct types of cells viz, neurons, astrocytes and oligodendroglia, all with different replicative schedules (Korr, 1980). It is therefore, possible that DNA polymerase in these cell types may vary depending upon the age of the brain. We have measured
the DNA polymerase activity in these cell types isolated from the rat brain at different stages of the lifespan. Effort was also made to distinguish between α and β polymerase activity making use of specific inhibitors.

MATERIALS AND METHODS:

Highly polymerized calf thymus DNA, dATP, dGTP, and dTTP were purchased from Sigma Chemical Company, St. Louis, MO, USA. Dideoxythymidine 5'-triphosphate was from P.L. Biochemicals Inc., Wisconsin, USA and (methyl-3H) dTTP (sp. act., 46 Ci/m mole) was from Radiochemical Centre, Amersham, England. Aphidicolin was a gift from Dr. A.H. Todd of I.C.I.Ltd., U.K. Polysaccharide purified from Physarum polycephalum was a kind gift from M. Shioda, University of Tokyo, Japan. All other chemicals used were of analytical grade.

Neuronal, astroglial and oligodendroglial cell enriched fractions were isolated as described in the chapter II.

Preparation of the Enzyme extract:

The homogenates of isolated cell enriched fractions were prepared by homogenizing the isolated cell pellets in homogenization medium, which consisted of 0.02 M Tris-HCl buffer, pH 7.5, 0.1 mM β-mercaptoethanol, 1 mM MgCl₂, 0.1 mM EDTA, 5% glycerol, 1% Triton X-100, and 0.5 M KCl. After the homogenization the samples were kept at zero to 4°C for one hour to aid the extraction of the polymerases from the nuclei and centrifuged at 100,000 xg for 1 hr. The clear supernatant thus obtained was used
as the enzyme source of DNA polymerase. An aliquot of the supernatant was taken for the protein estimation according to Lowry's method (1951).

**DNA polymerase assay:**

The reaction mixture contained in a total volume of 50 μl: 40 mM Tris-HCl pH 8.0, 1 mM β-mercaptoethanol, 7.5 mM MgCl₂, 4 mM ATP, 5 μg of 'activated' DNA (Loeb., 1969), 0.1 mM each dATP, dGTP, dCTP and 25 μM of dTTP (1 μCi). Incubation was carried out at 37°C for 20 min. At the end of incubation, 0.4 mg of DNA was added as carrier and the reaction was stopped by adding 2 ml of cold 10% TCA. The samples were kept in ice for 10 min and centrifuged at 4000 rpm for 5 min. The precipitate thus obtained was washed thrice with 5% cold TCA and twice with 95% ethanol. The precipitate after washing, was dissolved in 0.1 ml of 0.05 M NaOH and aliquots were taken into scintillation vials containing 10 ml of Bray's mixture and were counted in Beckman LS 1800 liquid scintillation counter. The enzyme activity was linear up to 30 μg of enzyme protein. Specific activity was expressed as picomoles of dTMP incorporated into activated DNA per mg of protein per hour.

In order to distinguish the activity into α and β polymerase, specific inhibitors were used. Aphidicolin was added in the reaction mixture at a concentration of 50 μM with a simultaneous reduction of dCTP concentration to 5 μM. ddTTP was added at a concentration of 1 mM. Polysaccharide isolated from the slime mold *Physarum polycephalum*, (Shioda and Murakami-Muro-
fushi, 1987) was added at concentration of 0.5 mg/ml. Further increase in concentrations of these inhibitors had not resulted any increase in the extent of inhibition.

RESULTS AND DISCUSSION:

The specific activities of DNA polymerase in neuronal, astroglial and oligodendroglial cells obtained from rat brains of various ages are presented in Fig 5. It may be noted that at all the ages studied, neuronal cells possessed the highest polymerase activity. The activity in these cells decreased from 16th day of embryonic life to adult life (225 days), but no further decrease occurred thereafter. Eventhough there was a slight increase in the old age (>540 days), it is not significant. These values compare well with those already reported by Waser et al. (1979) and by Subba Rao et al. (1985). In both astrocytes and oligodendrocyte fractions the activity steadily decreased with age starting from a high level at the neonatal stage.

It is intriguing that cerebral neurons, with no capacity to divide during postnatal life, should exhibit higher activities of DNA polymerase at all the stages of lifespan as compared with the other cell types, viz, astrocytes and oligodendrocytes which are the cells known to retain their replicative capacity to a significant extent throughout life. It is known that there are at least three distinct DNA polymerases in mammalian cells; polymerase α,β and γ (Weissbach et al., 1975 and Scovassi et al., 1980). In the brain also similar type of diversity of DNA poly-
Fig 5. DNA polymerase in neuronal, astroglial and oligodendroglial cells of developing and aging rat brain. Each point represents the average of 4 at 16th day gestation and 6 experiments at all other ages, with bars indicating the variation.
merases was noticed. Studies from different laboratories have shown that the activities of polymerase α and β could be distinguished by using specific inhibitors (Enderberg et al., 1978; Ikegami et al., 1978; Ohashi et al., 1978; van der Vliet and Kwant 1978; Waqar et al., 1978). Thus, α-polymerase can be inhibited specifically by aphidicolin and β-polymerase can be inhibited by ddTTP. These inhibitors have been implicated in a number of studies to define a role for these polymerases. In a number of studies DNA polymerase β has been postulated to have a role in the repair of DNA (Waser et al., 1979; Pedrali-Noy and Spadari, 1980; Seki et al., 1980; Giulotto and Mondello, 1981; Spadari et al., 1982). Studies on the DNA polymerase α has produced conflicting results. Several studies implicated a role for polymerase α in the repair process (Berger et al., 1979; Ciarrocchi et al., 1979; Hanaoka et al., 1979; Snyder and Regan, 1982) while other studies assigned no such role (Pedrali-Noy and Spadari, 1980; Seki et al., 1980 and Spadari et al., 1982).

Recent studies using different inhibitors suggested that both polymerases are involved in DNA repair depending upon the damaging agent used and the dosage applied (Miller and Chinault, 1982; Miller and Lui, 1982; Cleaver, 1983; Dresler and Lieberman, 1983). The precise physiological role of polymerase γ which is shown to be similar to mitochondrial DNA polymerase is not yet clear (Hubscher, et al., 1977).

Therefore, we have made use of these two inhibitors to distinguish DNA polymerase α and β in different cell types. The effect of aphidicolin and ddTTP in neurons, astrocytes and
oligodendrocytes are presented in Tables 15, 16 and 17 respectively. A summary of the average percentage inhibition is presented in Table 18.

As it can be seen, in neurons the DNA polymerase activity at all the postnatal ages was inhibited more than 90% by ddTTP, with aphidicolin exerting no inhibition at all at 225 and >540 days of age. During the embryonic stages, however, ddTTP inhibited the activity by 70% only, while aphidicolin did so by 9%. These results are taken to indicate that at all the postnatal ages, most of the DNA polymerase activity in neurons is of the β-type. The picture was, however, different in the case of astrocytes and oligodendrocytes. In astrocytes, the maximum inhibition (95%) of activity by ddTTP was observed at 1 day old postnatal, pointing to the fact that at this stage the polymerase present is almost exclusively of the B-type. At the other ages the inhibition varied from 48 to 78% with a marginal effect by aphidicolin at the 16th day of gestation and 225 days. From the pattern of the inhibition by the two inhibitors in the oligodendrocyte fraction, it can probably concluded that while B-polymerase is the predominant one throughout the postnatal life, some amount of α-polymerase also seems to be present at all the ages studied. There were few oligodendrocytes in the prenatal brain and therefore could not be isolated.

In view of the claimed specificities of the two inhibitors ddTTP and aphidicolin towards DNA polymerase β and α respectively, it can normally be expected that the sum total of inhibition by ddTTP and aphidicolin should reach close to 100%. How-
**TABLE 15**

**EFFECT OF APHIDICOLIN AND ddTTP ON DNA POLYMERASE ACTIVITY IN NEURONS OF RAT BRAIN AT DIFFERENT AGES**

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>ddTTP</th>
<th>% inhibition</th>
<th>Aphidicolin</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>16th day</td>
<td>2767.56</td>
<td>825.03</td>
<td>70.18</td>
<td>2506.38</td>
<td>9.44</td>
</tr>
<tr>
<td>(Prenatal)</td>
<td>294.34</td>
<td>142.08</td>
<td>61.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>2713.00</td>
<td>210.03</td>
<td>92.25</td>
<td>2630.00</td>
<td>3.00</td>
</tr>
<tr>
<td>(Postnatal)</td>
<td>341.80</td>
<td>108.00</td>
<td></td>
<td>345.80</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>449.23</td>
<td>34.55</td>
<td>92.30</td>
<td>531.43</td>
<td></td>
</tr>
<tr>
<td>(225 days)</td>
<td>81.47</td>
<td>16.31</td>
<td></td>
<td>171.57</td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>500.52</td>
<td>35.37</td>
<td>93.03</td>
<td>522.02</td>
<td></td>
</tr>
<tr>
<td>(&gt;540 days)</td>
<td>110.89</td>
<td>14.75</td>
<td></td>
<td>137.00</td>
<td></td>
</tr>
</tbody>
</table>

Activities are expressed as **picomoles** of TMP incorporated into activated DNA/mg of protein/hr.

Values are expressed as Mean + SD.

Numbers in parentheses represent number of experiments carried out.

For other details please see the text.
TABLE 16
EFFECT OF APHIDICOLIN AND ddTTP ON DNA POLYMERASE ACTIVITY IN ASTROCYTES OF RAT BRAIN AT DIFFERENT AGES

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>ddTTP</th>
<th>% inhibition</th>
<th>Aphidicolin</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>16th day</td>
<td>1476.84</td>
<td>442.46</td>
<td>70.04</td>
<td>1265.04</td>
<td>14.24</td>
</tr>
<tr>
<td>(Prenatal)</td>
<td>360.75</td>
<td>164.49</td>
<td>342.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>823.30</td>
<td>42.35</td>
<td>94.85</td>
<td>806.42</td>
<td>2.05</td>
</tr>
<tr>
<td>(Postnatal)</td>
<td>76.90</td>
<td>25.90</td>
<td>111.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>75.47</td>
<td>39.28</td>
<td>47.90</td>
<td>65.37</td>
<td></td>
</tr>
<tr>
<td>(225 days)</td>
<td>15.62</td>
<td>12.42</td>
<td>17.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>37.52</td>
<td>18.70</td>
<td>50.16</td>
<td>44.75</td>
<td></td>
</tr>
<tr>
<td>(&gt;540 days)</td>
<td>11.92</td>
<td>8.32</td>
<td>15.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Activities are expressed as **picomoles** of TMP incorporated into activated DNA/mg of protein/hr.

Values are expressed as Mean ± SD.

Numbers in parentheses represent number of experiments carried out.

For other details please see the text.
### TABLE 17

**EFFECT OF APHIDICOLIN AND ddTTP ON DNA POLYMERASE ACTIVITY IN OLIGO-DENDROCYTES OF RAT BRAIN AT DIFFERENT AGES**

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>ddTTP</th>
<th>% inhibition</th>
<th>Aphidicolin</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>245.10</td>
<td>91.51</td>
<td>62.60</td>
<td>205.00</td>
<td>16.36</td>
</tr>
<tr>
<td>(Postnatal)</td>
<td>53.19</td>
<td>10.35</td>
<td>44.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>144.43</td>
<td>28.10</td>
<td>80.50</td>
<td>137.42</td>
<td>4.80</td>
</tr>
<tr>
<td>(225 days)</td>
<td>42.30</td>
<td>10.70</td>
<td>41.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>78.80</td>
<td>44.20</td>
<td>43.90</td>
<td>74.63</td>
<td>5.29</td>
</tr>
<tr>
<td>(&gt;540 days)</td>
<td>16.30</td>
<td>18.35</td>
<td>28.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Activities are expressed as **picomoles** of TMP incorporated into activated DNA/mg of **protein/hr**.

Values are expressed as Mean ± SD.

Numbers in parentheses represent number of experiments carried out.

For other details please see the text.
TABLE 18
EXTENT OF INHIBITION BY ddTTP AND APHIDICOLIN IN DIFFERENT CELL TYPES
IN THE RAT BRAIN OF DIFFERENT AGES

<table>
<thead>
<tr>
<th>Cell type and inhibitor</th>
<th>Age</th>
<th>16th day of prenatal</th>
<th>1 day postnatal</th>
<th>225 days postnatal</th>
<th>&gt;540 days postnatal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ddTTP</td>
<td></td>
<td>70.2</td>
<td>92.3</td>
<td>92.3</td>
<td>93.0</td>
</tr>
<tr>
<td>Aphidicolin</td>
<td></td>
<td>9.4</td>
<td>3.0</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Astrocytes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ddTTP</td>
<td></td>
<td>70.0</td>
<td>94.9</td>
<td>47.9</td>
<td>50.2</td>
</tr>
<tr>
<td>Aphidicolin</td>
<td></td>
<td>14.3</td>
<td>2.0</td>
<td>13.5</td>
<td>Nil</td>
</tr>
<tr>
<td>Oligodendrocytes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ddTTP</td>
<td></td>
<td>62.6</td>
<td>80.5</td>
<td>43.9</td>
<td></td>
</tr>
<tr>
<td>Aphidicolin</td>
<td></td>
<td>16.4</td>
<td>4.8</td>
<td>5.3</td>
<td></td>
</tr>
</tbody>
</table>

Values represent average percentage of inhibition.

Number of experiments carried out are 4 at 16th day prenatal and 6 at all other ages.

For other details please see the text.
ever, this **is** not the case particularly, in **glial** fraction, as can be noticed in Table 18. This could be due to **two** reasons: 

1) the **a-polymerase** in crude extracts may not be so sensitive towards aphidicolin as it is reported to be in studies with pure a-polymerase enzyme (*Ikegami et al.*, 1978). 

2) the molecular species of a-polymerase present in the glial cells may be only marginally susceptible to aphidicolin. Indeed, there are reports in literature claiming the presence of multiple forms of DNA polymerase a in HeLa cells (*Pedrali-Noy* and *Weissbach*, 1977), calf thymus (*Hesslewood et al.*, 1978) and rat liver (*Holmes et al.*, 1974). On the other hand, the sensitivity of β-polymerase present in crude extracts towards ddTTP can not be suspected since at some ages profound inhibition by this inhibitor was observed. It thus appears that aphidicolin is not a good indicator to assess the levels of a-polymerase in the crude extracts as is the case in the present experiments.

Recently a polysaccharide isolated from the slime mold *Physarum polycephalum* was found to inhibit a-polymerase specifically with no effect on β-polymerase (*Shioda and Murakami-Murofushi, 1987*). We have therefore, studied the effect of this polysaccharide on the DNA polymerase activity in isolated cell enriched fractions from the rat brain of different ages. The results are presented in Table 19. It is clear from the results that polysaccharide inhibited the DNA polymerase activity by about 25% in astrocytes at all the ages studied, while this inhibition ranged from 30 to 50% in the oligodendroglial fraction. These results suggest that at all the postnatal stages,
### TABLE 19

**EXTENT OF INHIBITION BY POLYSACCHARIDE ISOLATED FROM PHYSARUM POLYCEPHALUM ON DNA POLYMERASE ACTIVITY IN NEURONAL, ASTROGLIAL AND OLIGODENDROGLIAL CELLS OF RAT BRAIN OF DIFFERENT AGES.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Neurons</th>
<th>Astrocytes</th>
<th>Oligodendrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (1 day old)</td>
<td>50.5 ± 4.7</td>
<td>25.8 ± 4.9</td>
<td>32.5 ± 5.8</td>
</tr>
<tr>
<td>Adult (6 Months old)</td>
<td>31.7 ± 5.0</td>
<td>27.7 ± 6.6</td>
<td>53.5 ± 5.3</td>
</tr>
<tr>
<td>Old (&gt; 540 days old)</td>
<td>23.5 ± 2.0</td>
<td>27.7 ± 3.0</td>
<td>32.3 ± 3.0</td>
</tr>
</tbody>
</table>

Values represent Mean of percentage inhibition + SD.

Numbers in parentheses represent number of experiments carried out.

Actual values (expressed as **picomoles** of TMP incorporated into activated DNA/mg protein/hr.) for neurons at young, adult and old ages are 2713, 449 and 500; for astrocytes 823, 75 and 37; for oligodendrocytes are 245, 144 and 78 respectively.

For other details please see the text.
about 25 to 50% of the DNA polymerase activity in the glial fraction is of α-type. It is surprising to see that polysaccharide inhibited DNA polymerase activity in neurons at all the three postnatal ages studied and this inhibition decreased with age (50% in young, 31% in adult and 23% in old). These results are not in complete agreement with our above mentioned inhibition studies with ddTTP and aphidicolin (Table 18). This could be due to marginal susceptibility of β-polymerase of neuronal cells to polysaccharide. Further work is necessary.

In any event, these results do indicate that rat cerebral neurons possess significant amounts of DNA polymerase activity both in adult and old life and that the enzyme is almost exclusively of β-type. On the other hand, the glial cells at adult and old stages of life seem to possess other type(s) of DNA polymerase in addition to the predominant β-polymerase. This appears to be in line with the known proliferative capacity of the glial cells in later stages of lifespan.