Effects of High Magnetic Fields on \textit{in vitro} Transcription of T7 and SP6 RNA Polymerases*

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Motivation via work on whole plants

The “Working” Hypothesis

Transcription and T7 Structure

Results with T7 and SP6

Future Directions

* Supported, in part, by the NSF via the NHMFL, DMR-0305371 (MWM), and NASA grant NNA04CC61 (ALP and RJF).

† NHMFL Research for Undergraduates (REU) Summer 2005.
TAGES - Plant Biomonitor of Microgravity

An Etruscan deity who possesses wisdom. He appeared from a groove when a field was newly ploughed and taught the gathered Etruscans the skills of divination and augury.

http://www.christusrex.org/www1/vaticano/ET2-Etrusco.html

- Why Arabidopsis?


- Why Adh?

Questions for the STS-93 experiment:
Is there sufficient hypoxia in Flight to initiate a hypoxic response?
Is it really hypoxia that the plants see?
Adh-GUS Reporter Gene System

Anoxia
Hypoxia
ABA
Cold
Drought
Salt

β-Glucuronidase - GUS

Adh gene promoter

GUS coding region

Quantification and Localization (at the level of gene expression!)
GUS: $P = 0.001$ (not B or B$^2$)

Real-Time Quantitative PCR

The Quantitative Results (A.-L. Paul et al., MAP1 and to be published.)

The “Working” Hypothesis: Magnetic Alignment vs. Magnetophoresis

The magnetic energy (Bothner-By, 1996; Tjandra et al., 1997):
\[
E = -\frac{1}{2\mu_0} \mathbf{B}(r) \cdot \mathbf{\chi} \cdot \mathbf{B}(r)
\]

Variations from anisotropies of \( \mathbf{\chi} \) and \( \mathbf{B} \):
\[
\delta E = -\frac{1}{2\mu_0} \left\{ \mathbf{B}(r) \cdot \mathbf{\delta\chi} \cdot \mathbf{B}(r) + 2 \mathbf{B}(r) \cdot \mathbf{\Delta\chi} \cdot \mathbf{\delta B}(r) \right\}
\]

An order of magnitude comparison of the two effects:
\[
R \approx \frac{2 \Delta \chi \delta B}{\delta \chi B}
\]

Top of leaves and Bottom of roots: \((\delta B/B) \approx 5 \times 10^{-3}\)
Anisotropy of \( \mathbf{\chi} \) for biomacromolecules:
(Maret, Dransfeld, 1985; Tjandra et al., 1997) \(\delta \chi \approx 10^{-33} \text{ m}^3/\text{molecule}\)
(Valles et al., 1997) \(\Delta \chi \approx 10^{-29} \text{ m}^3/\text{molecule}\)

\(R < 10^{-2} \Rightarrow \text{Magnetic Alignment} > \text{Magnetophoresis}!\)
“Simplicity does not precede complexity, but follows it.” Alan J. Perlis
*(EPIGRAMS IN PROGRAMMING)*

Move from “complex” *in vivo* plants to a simple *in vitro* system!

Try *in vitro* transcription of a single process:

Use T7 Ribomax® Express *in vitro* Transcription Kit (Promega).

**Key elements:**

1. T7 RNA polymerase is widely studied, including structure.
2. Elements of kit are simple and pure (T7 RNA polymerase, DNA template, rNTPs, and buffer).
3. Protocol simple, and commercial kits available.
Results: T7 Reactions

T7 Electrophoresis Results

<table>
<thead>
<tr>
<th>Controls</th>
<th>4.5 Tesla</th>
<th>9.0 Tesla</th>
</tr>
</thead>
</table>

Time (min): 1 5 10 20 | 1 5 10 20 | 1 5 10 20

I(t) / I(t = 1 min)

Time (minutes)

Control
4.5 Tesla
9.0 Tesla

20 – 25 Tesla: “null result”?
Results: SP6 Reactions

SP6 Electrophoresis Results

<table>
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Time (min): 1 5 10 20 | 1 5 10 20 | 1 5 10 20

Surprise again: 9 T strong enough to perturb transcription!?

SP6 RNA polymerase structure: not yet determined
(but believed to be similar to T7 RNA polymerase).

SP6: 874 residues   T7: 883 residues
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**Future Extensions/Work:**

T7/SP6: additional data at more/higher magnetic fields & longer time! (magnetic field power law?)

Magnetic Anisotropy from Structure (Worcester, 1978; Pauling, 1979): calculate/measure/predict differences (via RNCs) ??? (RDC = residual dipolar couplings)

Simple to Complex: *E. coli* (living system amplifier)! ?

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