MICROBIOLOGY JOURNAL CLUB

A POLYMERASE III-LIKE REINITIATION MECHANISM IS OPERATING IN REGULATION OF HISTONE EXPRESSION IN ARCHAEA

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Pyrococcus furiosus

► Archaea
► Hyperthermophile
► Contain sequence independent binding proteins
  - Nucleoid proteins and histones
  - Apparently no chromatin
► Transcription is Eukaryotic-like
  - Similar to RNA polymerase II
  - TATA binding protein (TBP)
  - Transcription factor B (TFB)
► Only one published account of complete transcription of *P. furiosus* gene
RNA Polymerases in Eukaryotes

- Eukaryotes possess three types of DNA dependent RNA polymerases (I-III)
  - RNAP I – located in the nucleolus, transcribes most rRNA
  - RNAP II – transcribes mRNA and small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), micro RNA (miRNA)
  - RNAP III – 5S rRNA, tRNA, U6 snRNA, RNA component of RNase P, vault RNA (vRNA)

- Transcription initiation, elongation, and termination
Template-based Reinitiation of Transcription in Eukaryotes

- Transcription of a gene impacts subsequent transcription
- Bypassing initiation steps enhances rate of transcription
- RNAP III (class III genes) undergoes hyper-processive reinitiation

RNAP III – Reinitiation

► Transcriptional machinery assembles at promoter
► RNAP III is directed to the site
  ▪ Initiation, elongation, and termination occur
► Some level of the transcriptional machinery remains at initiation site
  ▪ This allows for subsequent reinitiation of RNAP III
  ▪ Perhaps through topology adjustments of DNA
► Result is increased rate of transcription

**In vitro Transcription Assays - Figure 1**

- hpyA1 gene (histone) cloned into pUC19
- linearized with PstI prior to *in vitro* transcription
- reaction mixture contains $^{32}$P labelled UTP
- 6% polyacrylamide urea gels and visualized
- Transcription at 80°C resulted in mostly run-off transcripts
  - Poor termination
- Transcription at 90°C resulted in nearly all transcripts terminated at T1
  - Good termination
- Increased transcription at 90°C
  - Lane 1 vs 4
In *vitro* Transcription Assays – Figure 2

- A minimum of 5 T’s are needed for effective termination at T1
- 90°C after 10 mins
- Introduction of GC bases inhibits termination at T1
Hairpin structures can assist in terminating transcription.

Base pair mutations eliminating hairpin upstream of T1 resulted in slightly lower amounts of transcripts terminating at T1:
- “paused” transcripts indicated by arrows

Little effect at lower temperatures:
- Likely hairpin effect may be more pronounced at lower temperatures

Authors conclude this hairpin does not impact transcription termination.
In vitro Transcription Assays – Figure 4a

- Competition experiments to determine if termination sequences impact reinitiation of transcription
  - In vitro transcription without CTP in reaction to form stable ternary complexes (RNAP, DNA, RNA) stalled at +25 site
  - Remove transcription factors and purify
  - Add same template with (run off 421 bp) and without termination signals and sequences of hairpin formation (run off 237 bp)
  - Complete reaction mix added – multiple round
  - Reaction mix lacking transcription factors – single round
- In both cases no additional RNAP is added to competition assay
- Subsequent transcription can only take place with RNAP already stalled at +25 site
**In vitro Transcription Assays – Figure 4b**

- No competitor added
- Transcription of templates 1 & 2 are similar at 80°C
  - Performed at 80°C because template 2 has reduced stability at 90°C
- ~3-fold increase of transcripts (run off?) after 15 min in multiple round assay with both templates 2
- Template 1 has termination at sites T1-T4
- MR = multiple round
  - Transcription factors added
- SR = single round
  - No transcription factors added
**In vitro** Transcription Assays – Figure 4c

- Release of RNA from templates 1 & 2
- Stalled ternary complexes purified and the supernatant containing released RNA
  - At 80°C
- Majority of RNA from template 2 occurs in the supernatant (released) after 5, 10, & 15 min
- More RNA from template 1 is present in ternary complex compared to template 2 at 10 min
  - 42% vs 11%
- Authors demonstrate that template 1, containing terminator, exhibits delay in transcript release
**In vitro Transcription Assays – Figure 4d**

- **Competition assays**
  - Purified ternary complexes as before with multiple rounds approach
  - Transcription factors added
  - Addition of competitive template

- **Authors assume that RNAP is released similar to RNA released (figure 4c)**

- **Preference for template 1 after initiated on template 1 in the presence of template 2**
  - Left panel

- **Approximately equal affinity for templates 1 & 2 after initiated on template 2 in the presence of template 1**
  - Right panel

- **At 80°C**
In vitro Transcription Assays – Figure 4e

- Competition with template 3 lacks terminator
- At 80°C & 90°C
- After 10 min, analyze transcripts
- Template 1 is preferred over template 3 at both temperatures when initiated with template 1
In vitro Transcription Assays – Figure 5

- Promoter from *gdh* was fused to terminator from *hpyA1*
  - Transcript levels were determined after 10 min at 80°C and 90°C
- Template 3 has approximately the same transcription at both temperatures
- 2.5 fold increase in transcript yield from $P_{gdh}$ when fused to terminator of *hpyA1*
  - B lane 2 (0.2) vs A lane 4 (0.5)
- 2.4 fold increase for *hpyA1*

- *hpyA1* has predicted heat shock promoter
- Template 3 (lacks terminator) has high transcription at 80°C and 90°C
- *gdh* has reduced transcription at 90°C
**In vitro** Transcription Assays – Figure 6

- Termination efficiency impacts transcription rates
  - Introduction of AT mutations between T1 & T2 increased termination efficiency
  - Introduction of GC mutations between T1 and T2 lowered termination efficiency, slightly, compared to WT
- Both AT & GC mutations introduced between T1 & T2 reduced total transcript levels
- Authors conclude sequences in terminator region are critical for recycling of RNAP
Conclusions

- *hpyA1* gene has increased transcription & termination at T1 at 90°C
  - Minimum of 5 T’s are needed for effective termination at T1
  - Upstream hairpin structure of T1 does not impact termination
- RNAP exhibits delayed release from termination signal of *hpyA1* at 80°C
- RNAP prefers reinitiation with template containing effective terminator
- Terminator of *hpyA1* can improve transcription of promoter from *gdh* at 90°C
  - This increase in transcription is dependent on efficient termination
- Too much termination (as with AT mutant) results in less transcription
- Too low termination (as with GC mutant) results in less transcription
- Indicates that RNAP is recycled from terminator region to promoter of *hpyA1* during growth at 90°C
- Comparable to RNAP III reinitiation seen in eukaryotes