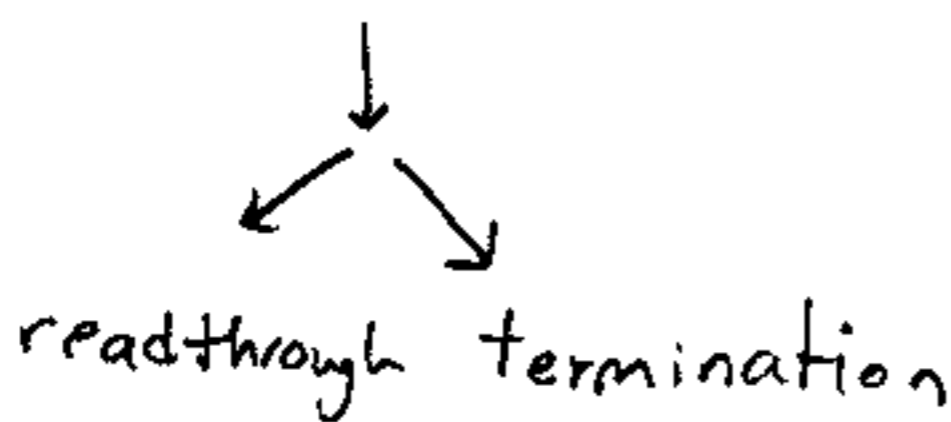


# Bob Landick: RNA polymerase Elongation in E. coli

2.3.97

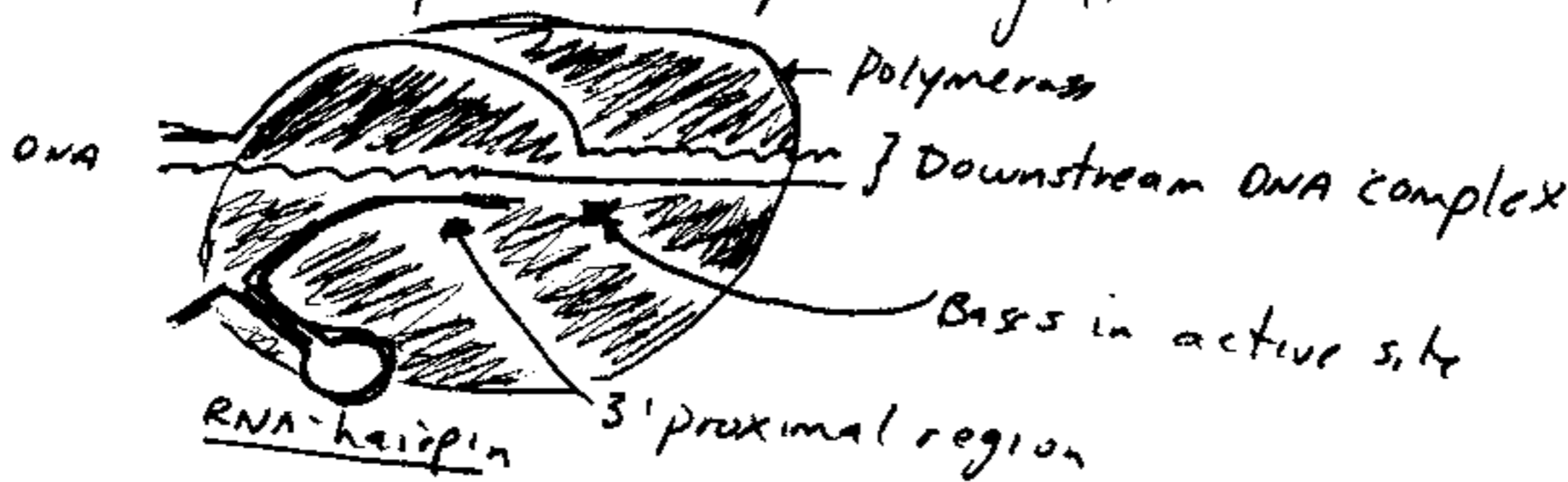
## Attenuation



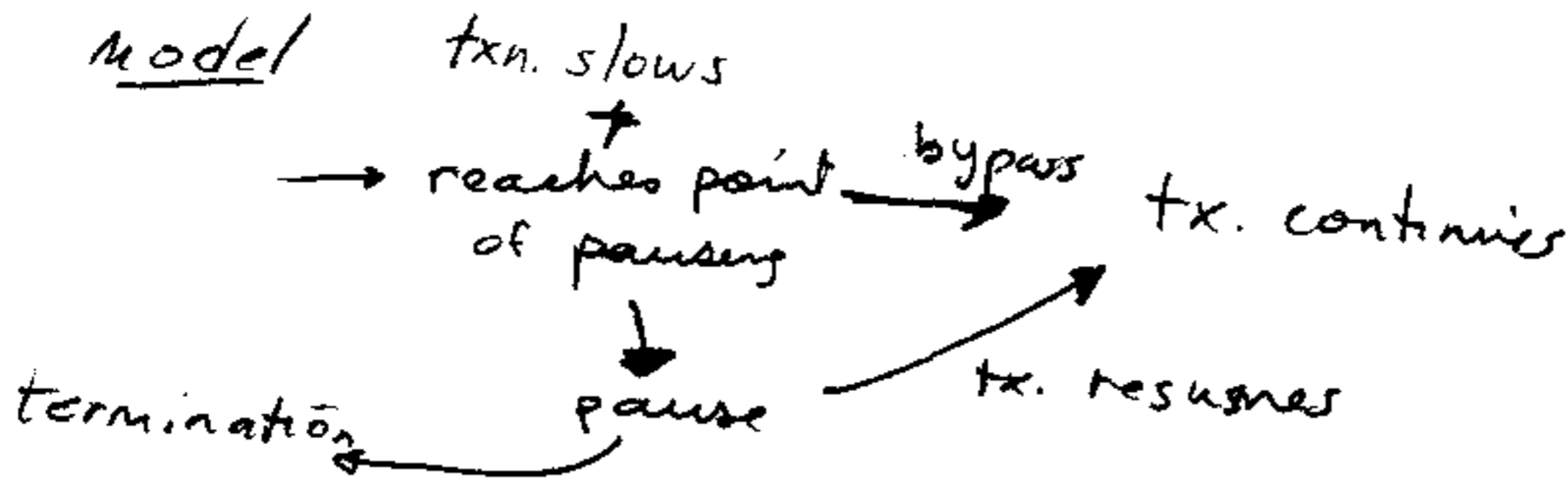
} Just one of many examples of regulatory information being fed into RNA polymerase.

## His biosynthetic pathway

- . Also subject to attenuation
- Four components to pause signal

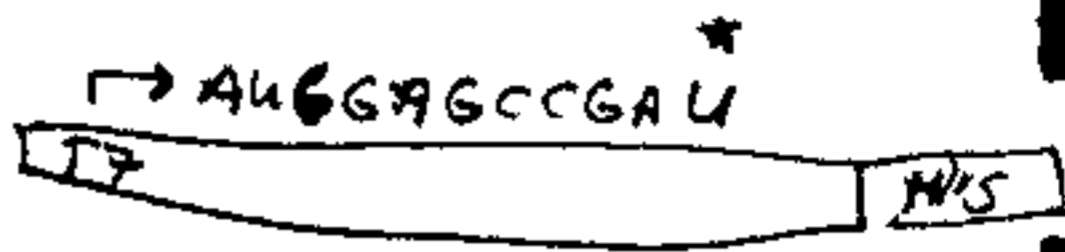


## Model



## Txn. pausing

- ① must synchronize everything  
- use UTP withholding
- ② calculate % of RNA polymerases that pause
- ③ calculate  $\lambda$  life of pause
- ④ compare effects of diff. mutations on pausing



## Polymerase in Action

Add 1 nucleotide at a time and then wash away and either purify complex or add another.

Exonuclease cleavage ... can map location of RNA pol. on DNA. Pause at site X, then cleave ... Pause at next site then cleave. This gave evidence for inchworm model, because as the pol. approached a pause site, the exonuclease protection zone did not change until right at pause site where it jumped forward ~10 bp.

## Footprinting RNA

- as ONA footprint shrunk... RNA footprint increased

### Models

Discontinuous  
Movement

Inchworm  
Movement

Sliding Clamp  
Movement

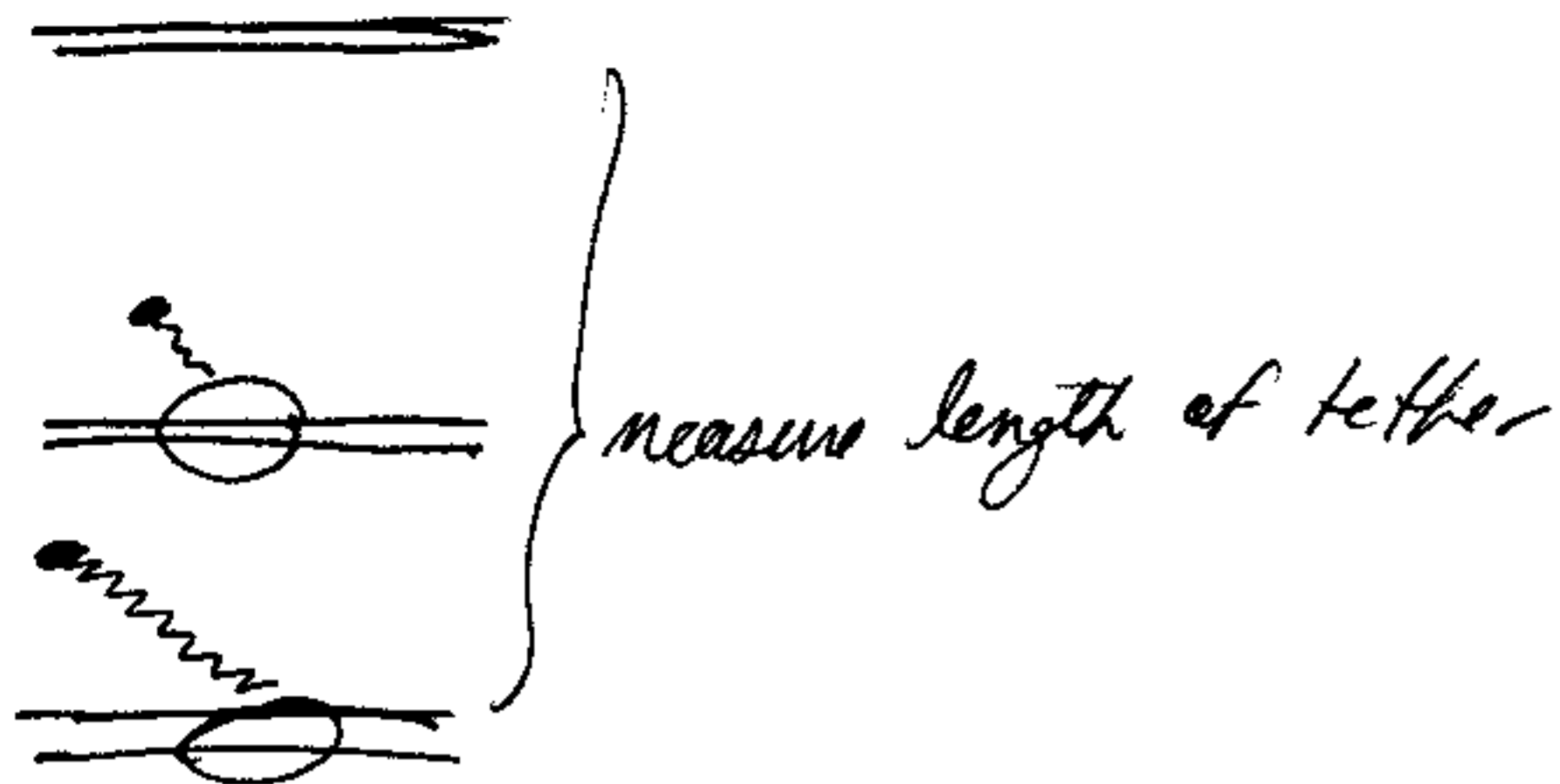
Can you use  
a different  
footprinting  
assay to  
distinguish the  
two models.

- can explain "inchworm"  
data, because it is  
the exonuclease sensitivity  
that determines the end result.

## Role of Downstream ONA & Hairpin

### Tethering

- Tether DNA
- Add ~~RNA~~ bead to RNA
- Can follow how close bead is to DNA



Can test different mutants

Mapping regions of pol B that interact with hairpins

- 15 aa region (909-952)  
<sup>between</sup>

- appears to interact in region near funnel exit area for RNA.

Speculation/Future

① RNA pol II + HIV pause site

② where is 3' end of RNA

③ many substitutions in RNA pol in hamper region in vivo are lethal

- suggests in cell RNA pol involved in RNA folding



Site in pol II loop?  
ends?