Balanced branching in transcription termination

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The theory of stochastic transcription termination based on free-energy competition [von Hippel, P. H. & Yager, T. D. (1992) *Science* 255, 809–812 and von Hippel, P. H. & Yager, T. D. (1991) *Proc. Natl. Acad. Sci. USA* 88, 2307–2311] requires two or more reaction rates to be delicately balanced over a wide range of physical conditions. A large body of work on glasses and large molecules suggests that this balancing should be impossible in such a large system in the absence of a new organizing principle of matter. We review the experimental literature of termination and find no evidence for such a principle, but do find many troubling inconsistencies, most notably, anomalous memory effects. These effects suggest that termination has a deterministic component and may conceivably not be stochastic at all. We find that a key experiment by Wilson and von Hippel [Wilson, K. S. & von Hippel, P. H. (1994) *J. Mol. Biol.* 244, 36–51] thought to demonstrate stochastic termination was an incorrectly analyzed regulatory effect of Mg^{2+} binding.

The branching ratio of the termination process in gene transcription is balanced. In ρ-independent termination in prokaryotes, the case most thoroughly studied, conventional gel experiments performed *in vitro* find a fraction P of elongating RNA polymerase reading through the termination sequence with probability P typically midway between 0 and 1 rather than unmeasurably close to either extreme. P is different for different terminators and can be made to exhibit order-1 changes by perturbing the enzyme's structure or the reaction environment. This effect is astonishing from the standpoint of microscopic physics, because a stochastic decision to read through or not (1, 2) requires a competition of transition rates—quantities of inverse time—that must be nearly equal for the branching to balance. RNA polymerase, however, is more the size of a glass simulation than of a small molecule and thus possesses a broad spectrum of natural time scales spanning many orders of magnitude. Without some physical reason for a particular scale to be preferred, rate competition ought to be severely unbalanced, meaning that one event occurs essentially always and the other never. Balanced branching in termination has been implicated in transcription regulation in a few cases (3, 4), but its broader regulatory significance and especially its robustness are still mysteries.

In this paper we examine the experimental facts relevant to the physical nature of termination with the goal of determining what, if any, principle selects the time scale to make the rates balance. Our conclusion is both surprising and unsettling. We find no evidence for such a principle, but do find glaring weaknesses in the case for stochasticity, and a large body of unexplained experimental results pointing to a termination decision that is partially deterministic.

Termination Efficiency

The simplest termination sequences are the ρ-independent terminators of prokaryotes—those that are capable of causing polymerase to terminate *in vitro* in the absence of the ρ protein factor. A representative sampling of these is reproduced in Table 6, which is published as supplemental data on the PNAS web site, www.pnas.org. This differs from lists that have appeared in the literature before (5, 6) by having been rechecked against the fully sequenced genome [*E. coli* K-12 MG 1655, ftp://ncbi.nlm.nih.gov/genbank/genomes/bacteria/Ecoli/ecoli.ptt (1999); ftp://ncbi.nlm.nih.gov/genbank/genomes/bacteria/Ecoli/ecoli.fna (1999); and http://www.ncbi.nlm.nih.gov/BLAST (1999)] and expunged of "theoretical" terminators identified only by computer search. They conform for the most part to the motif of a palindrome of typically 10 bp followed by a short poly(T) stretch (although there is tremendous variety in the length and composition of the palindrome), variation in the length of the poly(T) stretch, and occasional extension of the palindrome to include the poly(T) stretch. This enormous variability contrasts with the simplicity of stop codons, which terminate protein synthesis by ribosomes and have no other function.

ρ-independent terminators are characterized by "efficiencies," i.e., the fraction of assayed transcripts that terminate. These efficiencies rarely take on extreme values close to 1 or 0 when measured *in vitro*. In cases where a measurement *in vivo* exists as well, the latter is usually larger (7) and is occasionally unmeasurably close to 1. Balanced termination efficiency is commonly observed *in vivo* as well, however. Table 1 shows results from a particularly careful study (7) *in vitro* in which termination probabilities in *Escherichia coli* for wild-type terminators, mutant terminators, phage terminators (8), and terminators from *Shigella boydii* were measured under identical conditions. Despite the great variety of these sequences, the termination efficiency runs only from 2% to 88%. Many other researchers report similar values for terminators in *E. coli* and other bacteria (9), including artificially altered terminators (10). The results in Tables 2 and 3 show balanced termination for modified versions of the phage terminator *tR2* (11) and for mutant polymerase (12). These modifications make order-1 changes to the efficiencies themselves. Other researchers (9, 13) reported similar effects with different mutant polymerases. Modifications up to 20 bp upstream and downstream of the terminator cause significant changes to the efficiencies but without causing them to unbalance (14), as do large changes in monovalent and divalent ion concentrations (7), addition of NusA (11), temperature variations (15), and supercoiling (7). Thus, balanced termination efficiency is the norm, not the exception.

This finding requires explanation. The idea that a miracle of evolution causes this phenomenon is quite unsatisfactory because it is too robust. Not only are balanced efficiencies ubiquitous in nature, they *stay* balanced under conditions (such as those described above) that lie far outside those in which the organism lives. Moreover, one would be hard-pressed to construct any microscopic model capable of making such delicate decisions on the one hand and resisting stress from the environment on the other. The reason is that no known physical principle "protects" the behavior and enables it to occur reliably under a broad range of circumstances (i.e., enables it to be robust). However, there is a potential explanation that does not have this difficulty: The effect might be caused not by rate balance but by *state* balance. If polymerase possessed a small number of metastable configurational states and terminated deterministically depending on which state it was in, then

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Table 1. Termination efficiencies measured *in vitro* (8)

Sequence	Name	% T
GGCTCAGTCGAAAGACTGGGCCTTTCGTTTTAAT	*rrnB* t_1	84 ± 1
TCAAAAGCCTCCGACCGGAGGCTTTTGACTATTA	*tonB t*	19 ± 1
CCAGCCCGCCTAATGAGCGGGCTTTTTTTTGAAC	*trp a*	71 ± 2
CCAGCCCGCCTAATGAGCGGGCTTTTGCAAGGTT	*trp a 1419*	2 ± 1
CCAGCCCGCCTAATAAGCGGGCTTTTTTTTGAAC	*trp a L126*	65 ± 4
CCAGCCCGCCTAATAAGCGGACTTTTTTTTGAAC	*trp a L153*	8 ± 4
CTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTA	$T7T_e$	88 ± 2
GGCTCACCTTCACGGGTGAGCCTTTCTTCGTTCX	$T3T_e$	14 ± 2
GGCCTGCTGGTAATCGCAGGCCTTTTTATTTGGG	*tR2*	49 ± 4
AAACCACCGTTGGTTAGCGGTGGTTTTTTGTTTG	*RNA I*	73 ± 4

The first three terminators are native to *E. coli*. These are followed by three mutants, three phage terminators (9), and one from *S. boydii*. Far-right underlined sequences are termination zones.

balanced branching would become a simple, automatic consequence of thermalized state populations that required no physical miracles. Microscopic models that do this are easy to construct. The states in question would need to be metastable on the order of seconds to minutes because that is the time scale on which termination occurs (16), so that significant interconversion between terminating and read-through states did not occur during the termination process. Although times of this order are physically plausible, the experimental evidence for them in the biological context is complex and controversial. There is at present no consensus about what is and is not reasonable.

Large Molecules and Glasses

Such effects also occur in nonliving matter, however, and have been studied extensively there. In this context there is no disagreement. It is well established that hysteresis and metastability occur naturally and commonly in systems that are *large*. This occurrence is not an accident but rather one of the more celebrated examples of P. W. Anderson's "More Is Different" principle, the idea that large systems are often qualitatively different from small ones (17, 18). Because there is no reason for these principles to stop working in life, we find it natural that enzymes, which are extremely large by most measures of microscopic physics, should exhibit such behavior and *un*natural that they should not.

The strongest experimental precedents for mesoscale metastability in nature come from glasses, which may be thought of as very large molecules. All glasses have linear specific heats at low temperatures (unlike the T^3 behavior of crystals; refs. 19–21), stretched-exponential time dependence in response to perturbations [i.e., of the form $\exp(-At^{\beta})$ with $\beta < 1$, indicating a broad spectrum of decay rates rather than just one], and memory effects, such as "remanence" in spin glasses (22) or the well known failure of ordinary silica to crystallize without annealing.

Table 2. Termination efficiencies for modified versions of the phage λ terminator *tR2* (12)

Sequence	Name	% T
GTTAATAACAGGCCTGCTGGTAATCGCAGGCCTTTTTATT	*tR2*	40
GTTAATAACAGGGGACGTGGTAATCCGTCCCCTTTTTATT	*tR2-6*	56
TAATAACAGGCCTGGCTGGTAATCGCCAGGCCTTTTTATT	*tR2-11*	54
CCGGGTTAATAACAGGCCTGCTTCGGCAGGCCTTTTTATT	*tR2-12*	69
CGGGTTATTAACAGGCCTCTGGTAATCGAGGCTTTTTATT	*tR2-13*	11
ATAACAGGGGACGTGGTAATCGCCAGCAGGCCTTTTTATT	*tR2-14*	20
GTTAATAAAAGGCCTGCTGGTAATCGCAGGCCTTTTTATT	*tR2-16*	36
GGTTCTTCTCGGCCTGCTGGTAATCGCAGGCCTTTTTATT	*tR2-17*	67

Far-right underlined sequences are termination zones.

Table 3. Termination efficiencies for wild-type *E. coli* polymerase (rpo+) and mutant polymerase (rpo203) (13)

Sequence	Name	rpo+	rpo203
GCAACCGCTGGGGAATTCCCCAGTTTTCA	*trpC 301*	0	20
AACCGCTGGCCGGGATCGGCCAGTTTTCA	*trpC 302*	8	35
CAGCCGCCAGTTCCGCTGGCGGCTTTTAA	*trp t*	25	45
ACCAGCCCGCCTAATGAGCGGGCTTTTGC	*trp a 1419*	3	35
CAGCCCGCCTAATGAGCGGGCTGTTTTTT	*trp a 135*	65	80

trp t is native to the genome. The rest are either mutants or synthetic.

There is abundant evidence that large enzymes and proteins often possess "glasslike" properties of hysteresis, metastability, a broad spectrum of relaxation times, and memory. Crystals of myoglobin, a protein with a molecular weight of only 17,000, have linear specific heats at low temperatures (23) and exhibit stretched-exponential responses to photodissociation pulses (24). Denatured proteins refold on a variety of time scales ranging from nanoseconds to seconds (25). Permanent misfolding of proteins with molecular weights of only 30,000 has been implicated in prion diseases (26).

Many enzymes exhibit hysteresis in their catalytic rates (27, 28). The activity of cholesterol oxidase of *Brevibacterium* spp., a protein with molecular weight 53,000, was recently shown by fluorescence correlation techniques to have a memory effect persisting approximately 1 sec under normal conditions at room temperature (29). Other notable examples include wheat germ hexokinase [M_r of 50,000 (30)] with a half-life of 2 min (31), rat liver glucokinase [M_r of 52,000 (32)] with a half-life of 1 min (27), and yeast hexokinase (M_r of 50,000) with a half-life of 1–2 min (33). Thus, RNA polymerase complexes, which have a molecular weight of 379,000 and are comparable in size to the largest computer simulations of glasses ever performed, are good candidates for systems that exhibit glassy behavior.

Getting the experimental conditions right is often a prerequisite for observing glassiness in enzymes. Mnemonic behavior can be destroyed by "desensitizing" the enzyme with contaminants (30). Time scales can depend on enzyme, substrate, product, activator, and effector ligand concentrations as well as pH, buffers, and temperature (27, 33, 34). Memory effects can even depend on the order in which reactants are added: the mnemonic effect in yeast hexokinase occurs when it is preincubated with MgATP and free Mg^{2+} and the reaction is started with glucose, or preincubated with glucose and free Mg^{2+} and started with MgATP, but *not* if the enzyme is preincubated with glucose and metal-free ATP and then started with Mg^{2+} (27). Before hysteresis and memory effects were recognized, early investigators generally adjusted such reaction conditions until the "improper" behavior was eliminated (27).

Polymerase States

Although the size of RNA polymerase makes it plausible to expect glassy behavior on purely theoretical grounds, several direct lines of evidence indicate that the enzyme possesses a variety of metastable conformations that are influenced by the DNA template over which it passes and general experimental conditions:

(*i*) Polymerase has a catalytic mode distinct from RNA synthesis, as it can cleave the RNA transcript through hydrolysis (rather than pyrophosphorolysis, the reverse reaction of RNA synthesis; ref. 35), with the cleavage reaction requiring Mg^{2+} (35) being template-dependent (36) and stimulated by GreA and GreB proteins (37, 38) and by high pH (8.5–10.0; ref. 39). The last effect was discovered serendipitously, going unobserved for

decades because assay conditions were being optimized to maximize elongation rates, which occur at lower pH values [7.8–8.2 (40)] (39).

(*ii*) RNA polymerase mobilities in nondenaturing electrophoresis gels show significant and discontinuous variance while bearing nearly identical transcripts or identical-length transcripts with different sequences (41). These mobility variances are still observed if the RNA transcript is first removed by ribonuclease digestion (42).

(*iii*) RNA polymerase ternary complexes vary greatly in their stability and mode of binding to DNA (ionic or nonionic) in a template-dependent manner. Some complexes are stable against very high salt concentrations ($[K^+]$ = 1 M), whereas others (specifically those proximal to an upstream palindrome sequence) are salt-sensitive (completely dissociating in concentrations as low as 20 mM $[K^+]$). However, the salt-sensitive complexes are stabilized by millimolar concentrations of Mg^{2+} (43).

There is also direct evidence that these states affect the enzyme's propensity to elongate in a hysteretic, template-dependent manner, greatly complicating any attempt to achieve a delicate balance between elongation and termination rates:

(*i*) An elongating polymerase's Michaelis constants K_S for NTPs vary over 500-fold for different DNA template positions (44), and for different templates (45), although these effects are not observed for synthetic dinucleotide polymer templates (45).

(*ii*) The rate of misincorporation at a single site for which the correct NTP is absent is significantly different before and after isolation of ternary complexes (46).

(*iii*) Stalling elongating polymerase complexes (via nucleotide starvation) and then restarting them by nucleotide addition perturbs pausing patterns 50–60 bp downstream (47).

(*iv*) Stalled polymerase gradually "arrests" (i.e., is incapable of elongating when supplied with NTPs), with the approximate half-time for arrest estimated at 5 min (43) and 10 min (48) for different DNA templates. The polymerase can continue elongating if reactivated by pyrophosphorolysis, so the enzyme is still functional (43). Even after undergoing arrest, crosslinking experiments show that the internal structure of polymerase gradually changes over the course of the next hour (48).

(*v*) Observations of single elongating RNA polymerase molecules show that they have two elongation modes with different intrinsic transcription rates and propensities to pause and arrest (49).

There is thus considerable evidence to support the notion that polymerase has different conformations, and that these states are sufficiently metastable to influence polymerase function for some distance along the DNA template from where the enzyme's "memory" gets set.

Thermal Activation

The idea that polymerase memory is potentially relevant to expression regulation is not new (45). It is implicit in the work of Goliger *et al.* (50) and Telesnitsky and Chamberlin (51) and even explicitly speculated by the latter in print. However, because of the experimental evidence supporting the stochastic model of termination (1, 2) and the widespread belief—unjustified, in our view—that proteins equilibrate rapidly, this suggestion generated little enthusiasm. A key experiment supporting the stochastic model by Wilson and von Hippel (15) is both historically important and typical, so it is appropriate that we consider it carefully.

Wilson and von Hippel promoted and stalled RNA polymerase 8 bp upstream of the tR2 terminator hairpin of phage λ *in vitro*, thermally equilibrated at temperature T, and then launched it forward by adding NTP. The results are reproduced in Fig. 1*a*. Termination occurred at sites 7, 8, and 9 bp downstream of the beginning of the poly(T) stretch (Table 2) with probabilities P_7 $= N_7/N$, $P_8 = N_8/N$, and $P_9 = N_9/N$. The data were originally reported as a semilogarithmic plot of $1/\hat{P} - 1$ against temperature, where $\hat{P}_7 = N_7/N$, $\hat{P}_8 = N_8/(N - N_7)$, and $\hat{P}_9 = N_9/(N - N_7 - N_8)$. They concluded that all three branching probabilities $\hat{P}$ were thermally activated and had distinctly different activation energies. However, it is clear from Fig. 1*a* that this conclusion is false. The three probabilities P are essentially the same function and are well characterized by the

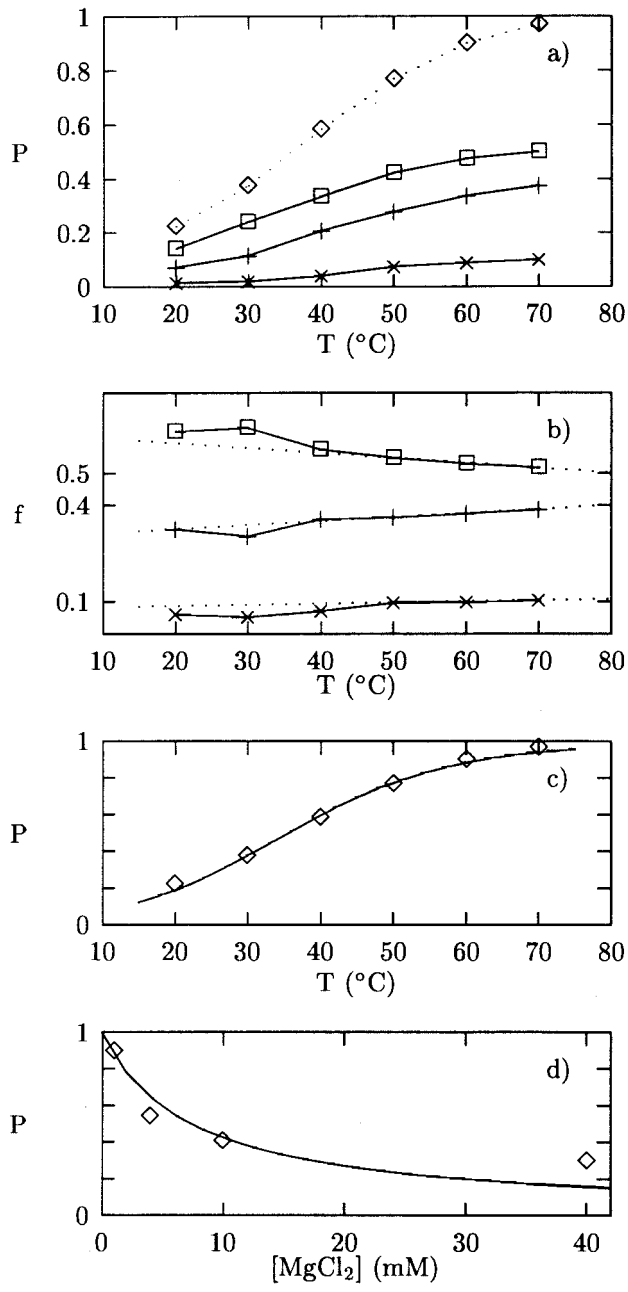

Fig. 1. (*a*) Temperature dependence of termination probability P for phage λ terminator tR2 reported by Wilson and von Hippel (15). +, □, and × denote the probabilities to terminate 7, 8, and 9 nt downstream from the beginning of the poly(T) stretch. The sum is shown as ◇. (*b*) +, □, and × above divided by ◇ to make a branching fraction f. (*c*) Comparison of ionization model Eq. **1** with ◇ from *A*. The ionization energy has been fit to ε_0 = 0.7 eV (16 kcals/mole; 1 cal = 4.18 J) and the quantity $n/M^{3/2}$ adjusted to make the curves match at 30°C. (*d*) Prediction of Eq. **1** for dependence on Mg^{2+} concentration compared with data of Reynolds *et al.* (7).

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sum $P = P_7 + P_8 + P_9$, also plotted in Fig. 1*a*. This is shown more explicitly in Fig. 1*b*, where the ratios P_7/P, P_8/P, and P_9/P are plotted against temperature. The flatness of these curves shows that the branching ratios among the three sites are essentially constant and independent of temperature within the error bars of the experiment. (Note that these fractions are also all of order 1.) Thus, the alleged spread in activation energies was an artifact of the plotting procedure.

Let us now consider the temperature dependence. It may be seen from Fig. 1*a* that P saturates to 1 at 80°C, the temperature at which Wilson and von Hippel report that the polymerase "will not elongate," i.e., has stopped working properly. This finding suggests that the effect has something to do with the overall enzyme state rather than with the termination process alone. Guided by this observation, we note that the activated behavior identified by Wilson and von Hippel is actually the formula for conventional monomolecular chemical equilibrium. The probability for a component with binding energy E_0 to be ionized off the polymerase is

$$P = \frac{1}{1 + Ae^{E_0/k_BT}[n]}, \qquad [1]$$

where $[n]$ is the concentration of this component and A is a slowly varying function of temperature. If one makes the approximation that A is a constant, then this reduces to the formula with which Wilson and von Hippel fit their data (15). That it works may be seen in Fig. 1*c*, where we plot the total termination probability from experiment against Eq. **1**, obtaining $E_0 = 0.7$ eV (1 eV = 1.602×10^{-19} J) by fitting it as well as A to match experiment at $T = 30$°C (52). Thus, reinterpreting this effect as an ionization equilibrium, we may account for the high-temperature intercept in the following way. In addition to the ionization state, the polymerase assumes different isomeric forms that terminate preferentially at sites 7, 8, or 9. (A glance at Table 2 will show that *something* besides the terminator structure itself determines the points at which termination occurs.) In the equilibration step, the polymerase molecules come to thermal equilibrium and a fraction P of them become ionized. All of these terminate at one of the three sites when launched. The rest read through.

A candidate for the ionizable component is an Mg^{2+} ion. In their studies of the effects of ion concentrations on termination efficiency, Reynolds *et al*. (7) discovered that Mg^{2+} has the unique effect of increasing termination efficiency to 100% for all terminators studied when reduced below 1 mM. The Mg^{2+} concentration in the experiments shown in Fig. 1*d* was 10 mM (15). Extrapolating at $T = 30$°C (52) using Eq. **1** we obtain, with no adjustable parameters, the fit to the $[MgCl_2]$ dependence found by Reynolds *et al*. (7) shown in Fig. 1*d*. The quality of this fit suggests that Mg^{2+} has a special function in regulating transcription, and that the temperature dependence in Fig. 1*a* is simply a thermal binding relation for this ion. This idea is corroborated by the recent structural studies of Zhang *et al*. (53) and others (54–56), who report that polymerase crystallized in the presence of 10 mM $MgCl_2$ has a Mg^{2+} ion bound at what appears to be the catalytic site of the enzyme.

There is evidence for more termination channels other than the ionization of Mg^{2+}. In Fig. 2 we reproduce results of Reynolds *et al*. (7), showing that terminator efficiencies tend to saturate at large Mg^{2+} concentration to values other than zero. The saturation values are balanced, and there is an evident tendency of them to cluster. Both effects are consistent with the polymerase executing an instruction at the terminator to read through conditionally, even when the ionizable component is bound, if its memory is appropriately set. There is obviously not enough data here to draw such a conclusion, however. We note that Reynolds *et al*. (7) also found order-1 effects on the termination efficiency from Cl^- and K^+, although with the opposite sign. The functions of these ions are not fully understood.

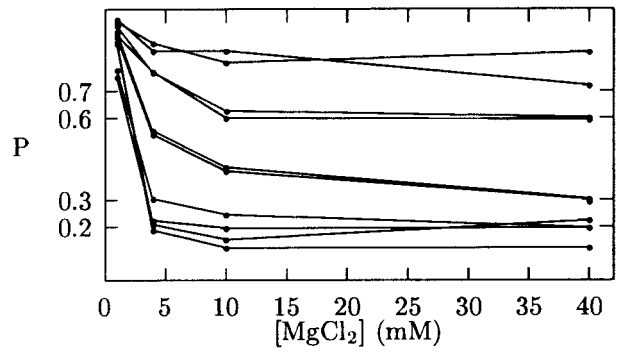

Fig. 2. Termination efficiency as function of $[MgCl_2]$ for 10 terminators, as reported by Reynolds *et al*. (7). The terminators are, top to bottom at the right edge, *RNA I*, *T7Te*, *rrnB T1*, *trp a L126*, *trp a*, *tR2*, *T3Te*, *P14*, *tonB t*, and *trp a L153*.

Antitermination

What experiments can detect internal memory? In general, one would look for cases in which polymerase acts differently under apparently identical conditions, suggesting an internal control mechanism of some kind. Such thinking motivates the following hypothetical experiment. One constructs a template with promoter P followed by two identical terminators (T_1 and T_2) and flanking DNA sequences in succession. If termination is stochastic, then the branching ratio at T_2 will be the same as that at T_1. If termination is deterministic and hysteretic, then the branching ratios will be different, depending on details. A passive termination at T_1 would result in no termination at T_2, because the polymerase that reads through has been "polarized," i.e., selected for the memory setting that codes for read-through. An active termination at T_1 would reprogram the memory there and cause a termination probability at T_2 different from that of T_1 but not necessarily zero. Variations of this design, e.g., adding more terminators, combining different terminators, changing their order, etc., could, in principle, answer more sophisticated questions, such as whether and how polymerase is reprogrammed in active read-through.

A few such experiments have already been performed on DNA templates containing antiterminators (sequences upstream of terminators that reduce termination efficiencies) and are thus less general than one would like, but they strongly support the idea of polymerase memory. There is indirect evidence in the case of N-antitermination of phage λ, the case most studied, that the memory is set by a physical attachment of the transcribed mRNA to the polymerase to form a loop (57). There is also evidence that this is not true generally (51).

Table 4. Sequences and corresponding termination probabilities at downstream *T7Te* and *trp a* for modified *lac* antiterminators reported by Telesnitsky and Chamberlin (52)

Sequence	*T7Te*	*trp a*
AATTGTGAGCGGATAACAATTTCACACAGGAAACAGGGAA	61	99
AATTGTGAGCGGATAACAATTTCACACAGGAAACAGAA . . .	51	52
AATTGTGAGCGGATAACAATTTCACACAGGAA . . .	73	99
AATTGTGAGCGGATAACAATTTCACGGAA . . .	45	99
AATTGTGAGCGGATAACAATTTCAGGAA . . .	71	99
AATTGTGAGCGGATAACAATTTCGGAA . . .	75	66
AATTGTGAGCGGATAGGAA . . .	88	75
No antiterminator	99	80

Table 5. Antitermination sequences *putL* and *putR* from the Hong Kong phage HK022 (60)

Sequence	Name
GAGCGCGGCGGGTTCAGGATGAACGGCAATGCTGCTCATTAGC	*putL*
GCGTGGTCAAGGATGACTGTCAATGGTGCACGATAAAAACCCA	*putR*

In 1989, Telesnitsky and Chamberlin (51) reported memory effects associated with the *lac* antiterminator found just downstream of the *Ptac* promoter in *E. coli*. [Goliger *et al*. (50) simultaneously reported similar findings.] Their key result is reproduced in Table 4. Insertion of *lac* 353 nt upstream of the terminator makes different order-1 modifications to the termination efficiencies of *T7Te* phage and *trp a*. The antiterminator contains a palindrome, and the antitermination effect is sensitive to modifications of the downstream 15-bp sequence. Three copies of *T7Te* placed in tandem downstream of *lac* showed that the antitermination effect is partially remembered through multiple terminators: the efficiencies were 44%, 60%, and 90%, but without the antiterminator they were 90%, >90%, and >90%, respectively.

King *et al*. (58) reported in 1996 that the *putL* and *putR* antitermination sequences of the Hong Kong phage HK022 (59), shown in Table 5, caused downstream read-through of a triple terminator consisting of tR′ from phage λ followed by the strong *E. coli* ribosome operon terminators *rrn B* t_1 and *rrn B* t_2. This effect was sensitive to the choice of promoter. When *putL* was inserted between the *Ptac* promoter and the triple terminator 284 nt downstream, and studied *in vivo*, the termination probability was 50%. Substituting the phage λ P_L promoter for *Ptac* under the same conditions resulted in complete read-through (although with wide error bars). When this experiment was repeated *in vitro*, the antitermination effect was found to be smaller and to persist through all three terminators. The read-through probabilities at tR′ were 34% and 31% for promotion by P_L and *Ptac*, respectively, but 57% and 27% for *rrnB* t_1, and 76% and 40% for *rrnB* t_2. This result is incompatible with statistical termination, for both the antitermination effect itself and the changes resulting from switching promoters are order-1 effects that do not add. They also reported that reduced Mg^{2+} concentration destroys the antitermination effect.

Conclusions

Antitermination and related effects do not prove the existence of long-lived polymerase isomers because there are other potential explanations, notably interaction with the transcript. They are nonetheless critically important as the class of experiment that most simply tests the physical issues raised by the phenomenon of balance in termination. If the decision to terminate is deterministic, then reading through a terminator *must* change the branching probabilities of other terminators placed downstream in characteristic ways. Although there are examples of such effects in the literature, they have not been studied in sufficient detail to determine whether they are rules or exceptions to rules. This paper's central prediction is that this effect should occur generally.

It must be emphasized that our identification of memory as important in termination is based on considerations of physics, not phenomenology. The balance in termination is too robust, in our view, to be caused by a delicate balance of stochastic transition rates. A molecule the size of RNA polymerase possesses a spectrum of natural time scales spanning 15 orders of magnitude. Without a physical principle that selects out one of these as preferred, rates should never compete in a balanced way. No microscopic model that can do this has ever been constructed, nor can it be, in our view. However, systems of this size exhibit hysteresis and memory very generally, and indeed only fail to do so if they crystallize with small unit cells. Thus, termination that is partly a deterministic decision based on the state of the polymerase is both physically reasonable and capable of accounting naturally for balance, provided that the number of states is small.

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