PERSPECTIVES: MOLECULAR BIOLOGY

Running the Red Light

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RNA polymerases are the molecular motors that transcribe the DNA template into messenger RNA (mRNA). As the RNA polymerase travels along the DNA, it forms an elongation complex from which the elongating mRNA chain emerges (see the figure). Molecular biologists have long wondered why RNA polymerase pauses at certain sites as it moves along the DNA and how transcription is halted when the enzyme encounters DNA sequences called terminators. Two reports, one by Davenport *et al.* on page 2497 of this issue (*1*), shed light on the mechanics and kinetics of RNA polymerase pausing (*1*) and termination (*2*), using video light microscopy to follow the progress of single elongation complexes during transcription. The success of these two studies demonstrates the value of biologists, physicists, and statisticians working together. These findings, together with earlier studies of elongation complexes in solution, support a nonequilibrium model of transcription (*3*).

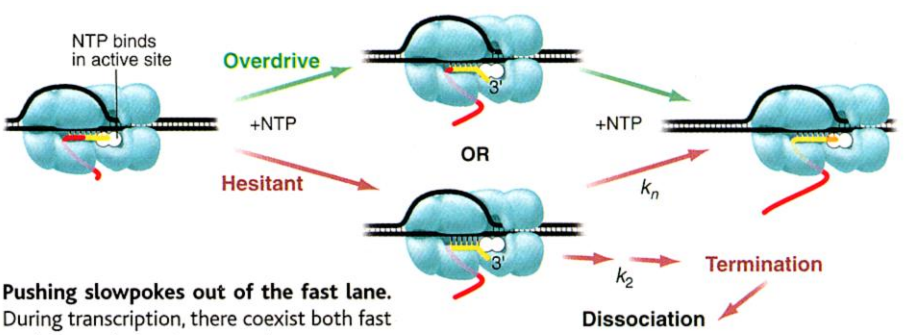
Transcription is usually followed by analyzing the time course of production of a radiolabeled intermediate (the elongating mRNA chain) as the DNA sequence is read by the enzyme. Sudden local decreases in the transcription rate manifest themselves as a transient accumulation of mRNAs of a certain length—these do not appear when RNA polymerase travels at its usual cruising speed without pausing or stopping. Biochemical analyses enable pause sites to be accurately pinpointed to within a single nucleotide of their location. The position of the enzyme, the mRNA product, and the DNA template during pausing can be established with similar accuracy. But the loss of synchronization in a solution of elongation complexes complicates the kinetic analysis of pausing and termination.

This drawback can be circumvented by following the behavior of a single elongation complex. In the original experimental design, elongation complexes were mounted on a glass surface and a bead was attached to the DNA downstream of the RNA polymerase (*4*). The absence of nucleotides caused the RNA polymerase to stall at a precise position on the DNA. When nucleotides were added back to restart transcription, the motion of the bead, as the DNA was threaded through the elongation complex, could be followed. This system has been used to assess the force (load) needed to be applied to the bead to counteract the motion of the RNA polymerase (*5*, *6*). Davenport *et al.* (*1*) modified this system so that they could both precisely adjust the counteracting force and decrease the damage inflicted on the elongation complex. This enabled them to record the relative displacement of DNA (with a resolution of about 20 base pairs) over distances of more than 2000 base pairs. From these measurements, they were able to accurately map pause and arrest sites on the DNA and to determine RNA polymerase cruising rates (the maximal speed reached between two pauses).

The fluctuations in cruising rates are surprisingly large and display a bimodal distribution. They do not seem to depend on the applied counteracting load or on the DNA sequence. Thus, there appears to be an intrinsic heterogeneity in the elongation complexes themselves, a possibility suggested by others (*7*–*9*). Also, the coexistence of slower and faster RNA polymerases might explain how regulatory proteins modulate the behavior of elongation complexes when they encounter terminator sequences (*10*–*12*). The study of individual elongation complexes provides direct evidence that RNA polymerases indeed have different intrinsic speeds. The next step is to assess the frequency with which speedier RNA polymerases convert to slower molecules and vice versa.

The skill in handling the results from single elongation complex experiments lies in finding clear correlations (or lack thereof) between specific subsets of data. For example, Davenport *et al.* observed that not all elongation complexes stop when they reach a pause site. This led them to suggest that a correlation exists between cruising rate and a tendency to pause. They demonstrate that tortoise elongation complexes pause more frequently than their hare brethren and that this tendency is independent of the counteracting force applied to the bead. Theoreticians are now faced with the challenge of explaining why the multiple energy barriers associated with the sequential movement of the elongation complex along the DNA are insensitive to the force applied to the DNA [(*6*) and references quoted therein].

In a parallel study, Yin *et al.* (*2*) observe the behavior of an elongation complex when it encounters a terminator sequence. They designed their assay such that the amplitude of the Brownian motion of the reporter bead varied depending on whether the elongation complex stopped at the terminator sequence or moved on through it. The investigators carefully compared their measurements on single elongation complexes with those obtained from parallel experiments on nonimmobilized complexes in solution. The results are clear-cut. When elongation complexes encounter a terminator sequence, they either pay no attention to the signal and add the next nucleotide to the growing mRNA chain, or they irreversibly pause resulting in release of mRNA and displacement of the RNA



Pushing slowpokes out of the fast lane. During transcription, there coexist both fast and slow elongation complexes—composed of DNA (black), RNA polymerase (blue), and the elongating mRNA chain (red and yellow). They are characterized by the variable stability of the DNA–RNA hybrid formed just upstream of the RNA polymerase catalytic site and by the different conformations of the proteins that clamp the hybrid into position. Slower, more hesitant elongation complexes are selected out of the transcription pathway by terminator sequences in the DNA (brown). As these slower complexes pause, the efficient formation of an RNA hairpin leads to dissociation of the three components, release of mRNA, and termination of transcription. Faster complexes bypass pause, arrest, and termination signals in the DNA (green), possibly in response to antiterminator proteins that stabilize the DNA–RNA hybrid (*11*). When an elongation complex encounters an ordinary pause site, a similar type of nonequilibrium competition takes place, but the formation of the RNA hairpin is less efficient and the mRNA is not released. NTP, nucleoside triphosphate; k_2 and k_n, rate constants. [Adapted from (*12*)]

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polymerase from the DNA. Time course experiments should allow putative intermediates in the termination pathway to be characterized (*13*).

Similar studies performed at the initiation of transcription reveal that proteins regulating transcription have two functions: to accelerate formation of the elongation complex, and to interact with and guide the complex along the DNA. Now it appears that at certain terminator sequences some regulatory proteins cause all of the RNA polymerases to behave in a similar way (*11*).

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RETROSPECTIVE

William Hamilton (1936–2000)

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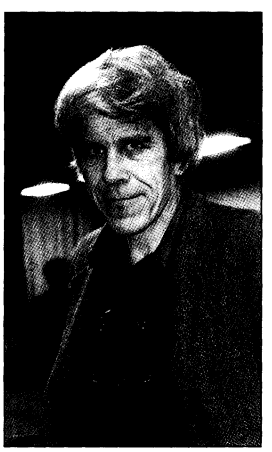
Bill Hamilton, one of the foremost evolutionary theorists of the 20th century, has died at the age of 63 from complications following malaria.

Bill is best known for the concept of inclusive fitness that he developed as a Ph.D. student at the London School of Economics. Hamilton's Rule, as it is known, is a form of genetic accounting that weights the fitness effects of an individual's actions on relatives according to their degree of kinship. Thus, from the perspective of the individual's genes, a benefit conferred on a sibling is four times the value of the same benefit conferred on a cousin but only half the value of the benefit conferred on the individual. This deceptively simple idea has revolutionized the way biologists think about the evolution of social behaviors.

Because he packed so much into each one, Bill's output of papers was small, but many have become foundation documents for new subdisciplines of evolutionary biology. The special quality of his papers was their unusual combination of a deep theoretical insight and a first-hand, encyclopedic knowledge of natural history. His article on extraordinary sex ratios proposed that many insects produce far more female than male offspring to reduce competition among brothers for mates. He introduced the idea of an unbeatable strategy and showed that this strategy differed for sex-linked and autosomal genes. Not only did this paper establish modern theoretical approaches to the evolution of sex ratios, but it also included early recognition of the potential for conflict among different elements of the genome.

Bill made significant contributions to understanding the evolution of senescence, dispersal, and cooperation among nonrelatives. The latter part of his career was devoted to exploring how parasites are involved in the maintenance of sexual reproduction. There is no better introduction to Bill's idiosyncratic world-view than his collected papers, published with autobiographical annotations under the title *Narrow Roads of Gene Land*. Bill had an enduring interest in Japan, and his title pays homage to Basho's *Narrow Roads of Oku*. In Basho's work, the traveling artist uses his experience to make poignant observations about human nature; in Hamilton's version, it is the wandering scientist who uses genetic and analytical reasoning to discover insights into animal behavior and evolution.



Bill was a lecturer at Imperial College, London, from 1964 to 1977; a Museum Professor at the University of Michigan from 1978 until 1984; then he returned to England as a Royal Society Research Professor at Oxford University. He received many honors, including the Darwin Medal, Kyoto Prize, and Crafoord Prize. The affection for him of those who knew him reflected his extraordinary humility, humor, and endless curiosity. He rarely came away from a seminar, no matter how poor, without a kind remark about an intriguing piece of natural history sometimes accidentally stumbled upon by the speaker.

Bill's interests ranged widely across disciplines, but natural history was his true passion. An Oxford boat trip would bring a stream of information, as he expertly wielded the punting pole, about the mating behavior of the midges buzzing overhead, the life history of the rust fungus infecting the nettles along the banks, or the diversity of brood parasites in the nests of bumblebees. But Bill would have been equally at home on the Amazon, discussing the aquatic caterpillars drifting by the gunwales, or the fruit-eating fishes foraging among the submerged crowns of rainforest trees in a seasonally flooded forest.

Bill constantly set himself challenges, such as learning to speak fluent Portuguese while working in Brazil, or cycling faster than any car encountered on the road between Wytham and Oxford. Personally, he regarded himself as "a lumbering dinosaur of the romantic movement," and spent several years writing a novel. It is a swashbuckling tale of intrigue, romance, diamond smuggling, gun-running, and exotic flora and fauna, not to mention a chapter in which the protagonist changes sex. His disregard for personal safety led to his last, fatal trip to the Congo in search of the origins of AIDS. Seniority and success never mellowed his fierce integrity and quest for truth. The parasites, which he believed played such an important part in evolution, at last brought an end to a life that had contributed so much.

Above all else, Bill loved insects. His letters and papers abound with tales of gall wasps, fighting stag beetles, Pharaoh's ants, and scarlet tiger moths. He once expressed a desire that when he died his body should be laid out in the Brazilian forest (adequately secured against larger scavengers such as possums and vultures) so that it could be buried by the great *Coprophanaeus* beetle. His poetic vision is a fitting epitaph (*1*): "They will enter, will bury, will live on my flesh; and in the shape of their children and mine, I will escape death. No worm for me or sordid fly, I will buzz in the dusk like a huge bumblebee. I will be many, buzz even as a swarm of motorbikes, be bourne, body by flying body out into the Brazilian wilderness beneath the stars, lofted under those beautiful and unfused elytra which we will all hold over our backs. So finally I too will shine like a violet ground beetle under a stone."

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