21. D. F. Stern *et al.*, *Mol. Cell. Biol.* **6**, 1729 (1986).
22. P. P. Di Fiore *et al.*, unpublished data.
23. K. Semba *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6497 (1985); J. Yokota *et al.*, *Lancet* **1986-I**, 765 (1986).
24. M.-C. Hung *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 261 (1986).
25. T. A. Libermann *et al.*, *Nature (London)* **313**, 144 (1985); Y.-H. Xu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7308 (1984); C. R. King *et al.*, *Nucleic Acids Res.* **13**, 8477 (1985).
26. D. J. Slamon *et al.*, *Science* **235**, 177 (1987).
27. F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973).
28. R. C. Mulligan and P. Berg, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2072 (1981).
29. C. Knicley provided excellent technical assistance.

1 December 1986; accepted 23 April 1987

Variable Occurrence of the *nrd*B Intron in the T-Even Phages Suggests Intron Mobility

JOAN PEDERSEN-LANE AND MARLENE BELFORT

The bacteriophage T4 *nrd*B gene, encoding nucleoside diphosphate reductase subunit B, contains a self-splicing group I intervening sequence. The *nrd*B intron was shown to be absent from the genomes of the closely related T-even phages T2 and T6. Evidence for variable intron distribution was provided by autocatalytic ^{32}P–guanosine 5′-triphosphate labeling of T-even RNAs, DNA and RNA hybridization analyses, and DNA sequencing studies. The results indicate the nonessential nature of the intron in *nrd*B expression and phage viability. Furthermore, they suggest that either precise intron loss from T2 and T6 or lateral intron acquisition by T4 occurred since the evolution of these phages from a common ancestor. Intron movement in the course of T-even phage divergence raises provocative questions about the origin of these self-splicing elements in prokaryotes.

WHILE SELF-SPLICING RNAs ARE remarkable for their autocatalytic properties (*1*), there is mounting evidence implicating these introns as mobile elements. There are several examples where the presence of eukaryotic group I introns is not uniform in equivalent genes of closely related species [for example, the large ribosomal RNA (rRNA) of *Tetrahymena* (*2*)] or even in different strains of the same species [for example, the large rRNA of *Saccharomyces cerevisiae* mitochondria (*3*)]. In the latter case site-specific transposition of the intron from intron$^+$ to intron$^-$ copies of the gene, with the aid of an intron-encoded endonuclease, has been demonstrated (*3*). Furthermore, there is evidence (based on conserved features of sequence and structure) for the transfer of group I introns between different fungal mitochondrial genomes (*4*) and between fungal mitochondrial genes and the *Tetrahymena* nucleus (*5*). Surprisingly, the protein potentially encoded by the intron open reading frame (ORF) of gene *td* in bacteriophage T4 (*6*) shares homology with intron ORFs of three group I mitochondrial intervening sequences of filamentous fungi (*7*). This observation led to speculations on a recent genetic exchange between these fungi and the genome of T4 (*7*).

In the face of these provocative observations that argue in favor of intron mobility, we have investigated the distribution of the group I *nrd*B intron of phage T4 (*8, 9*) in the T-even phage family. We provide evidence that the intron in the T4 *nrd*B gene, which encodes the B subunit of ribonucleoside diphosphate reductase, is absent from the genomes of T2 and T6. These results indicate the dispensability of the *nrd*B intron for T-even phage viability and imply movement of the T4 *nrd*B intron since the divergence of the closely related T-even phages from their common ancestor.

The existence of multiple T4 genes encoding group I self-splicing RNAs was inferred by labeling T4 RNA with ^{32}P–guanosine 5-triphosphate (GTP) under self-splicing conditions (*8*). In addition to a labeled band corresponding to the previously identified *td* intron (*6, 10*), several other labeled species were seen. The most intense of these other bands corresponds to the 0.6-kb *nrd*B intron (*8*). The profile of labeled bands was strikingly different in a similar autocatalytic ^{32}P-GTP labeling experiment with T2 and T6 RNAs (Fig. 1). While the *td* band appeared to be present in all three phages, the 0.6-kb *nrd*B species was apparently absent from T2 and T6 RNA. The *td* intron is indeed homologous throughout the T-even phages, as demonstrated by hybridization and dideoxy primer-extension analysis of pre–messenger RNA (mRNA) and splice products (*11*). In contrast, similar experiments argue in favor of the variable occurrence of the *nrd*B intron in the T-even phage family.

Dot-blot analysis revealed that several *nrd*B intron–specific probes that gave a strong hybridization signal with T4 DNA or RNA did not hybridize to T2 or T6 DNA or RNA (Fig. 2, probes 3 and 4). This was true for end-labeled oligonucleotide probes directed against the intron ORF (probe 4) as well as for probes that hybridized to noncoding sequences believed to form part of the active splicing conformation (probe 3). Similar results were obtained with a nick-translated intron probe (see legend to Fig. 2C). The *nrd*B intervening sequence thus appears to be absent from the genomes of T2 and T6. In contrast, the *td* intron-specific control probe hybridized throughout, except to a T4 phage construct that has a *td* deletion (Fig. 2A, probe *td*i). These data, suggesting the absence of the *nrd*B intron from T2 and T6, are in agreement with the results obtained with a synthetic

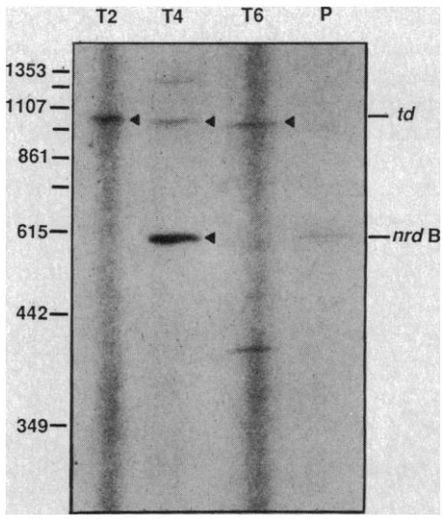

Fig. 1. Labeling of T2, T4, and T6 RNAs with ^{32}P-GTP. RNA was extracted from T2-, T4- or T6-infected *Escherichia coli* B 12 minutes after infection or from cells transformed with plasmid pJSK6 after transcriptional activation of the intron-containing *nrd*B gene (*8*). After incubating the deproteinized RNA with α-[^{32}P]GTP at 42°C for 1 hour under self-splicing conditions (*8, 18*), samples were separated on a 5% acrylamide gel alongside denatured DNA size standards (a 123-bp ladder) used to infer species lengths (left). The position of the *td* intron was indicated by a *td* control clone and that of the *nrd*B intron from the band in lane P, which contains labeled RNA from pJSK6. The absence of an *nrd*B band of equivalent intensity or mobility from the T2 and T6 lanes should be noted. Unmarked bands in the T4 and T6 lanes have not been identified and may well represent other group I introns.

Wadsworth Center for Laboratories and Research and School of Public Health Sciences of the State University of New York at Albany, New York State Department of Health, Albany, NY 12201.

oligodeoxynucleotide probe (25-mer) complementary to the T4 *nrd*B mRNA ligation junction (Fig. 2, probe 2). Whereas this probe hybridized to RNAs from all three phages and to DNA from T2 and T6, no signal was observed with T4 DNA. These are the predicted results for a split *nrd*B gene that is spliced at the RNA level in T4 and for an *nrd*B gene that is uninterrupted at the equivalent site in T2 and T6. These observations were confirmed by DNA sequence analysis of the T2 and T6 *nrd*B genes across the site that contains the intron insertion in T4 (Fig. 3). Clearly the T2 and T6 genes are contiguous at this site.

The T-even phages are similar by many criteria. In addition to complementation, recombination, and immuno–cross-reactivity between these morphologically similar viruses, there is extensive DNA sequence homology. Relatedness has been demonstrated over the entire length of the phage genomes (160 to 166 kb) by heteroduplex analysis, which indicates 88% homology (*12*), and by sequence analysis of selected homologous genes (*13, 14*), which in some cases reveal upward of 97% homology (*13*). Our data demonstrating the absence of the *nrd*B intron from T2 and T6 are consistent with the heteroduplex studies in which "bubbles" of nonhomology in the *nrd*B region were observed with T2/T4 and T6/T4 hybrids but not with T2/T6 hybrids (*12*). These findings suggest either horizontal intron acquisition by the uninterrupted T4 *nrd*B gene since its divergence from a common T-even ancestor, or intron loss from the ancestral *nrd*B gene by a precise excision event during the evolution of T2 and T6. In either event the results indicate the nonessential nature of the intron both in terms of *nrd*B gene expression and T-even phage viability.

The catalytic nature of RNA has fueled arguments that RNA was the primordial nucleic acid and that introns have been present since the earliest evolutionary times (*15, 16*). The relatively rare occurrence of intervening sequences in prokaryotes has been attributed to their loss under pressure to streamline these genomes in the interests of replicative efficiency (*16*). Despite their rarity, the existence of introns in prokaryotes lent credence to the antiquity of these elements (*16, 17*). While our results do not contradict the primeval nature of introns, their variable occurrence in homologous genes of closely related phages must be taken into account when considering the origin of these elements in prokaryotes. If indeed the group I introns are capable of lateral movement, it is possible that they have recently invaded genes of modern prokaryotes. Wherever the truth behind these observations may lie, the intron polymorphisms in the T-even phage genomes present us with a system to investigate the mobility of these elements in a prokaryotic context.

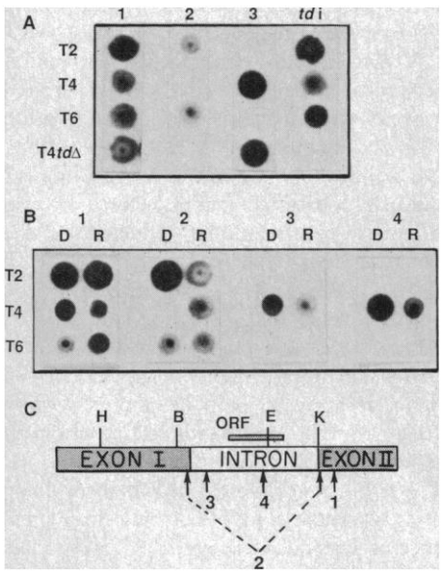

Fig. 2. Dot blot hybridization analysis of T-even phage DNA and RNA. (**A**) DNA blots. Aha III–digested, heat denatured (95°C, 3 minutes) DNA from T2, T4, T6, and T4*td*Δ, a construct deleted for a portion of the *td* gene including the intron, was applied to nylon membranes (100 ng per spot). These were probed with 5′-^{32}P-labeled oligonucleotides, directed to regions indicated in (C) of the figure. Probes were complementary to T4 *nrd*B exon II (probe 1), splice junction (probe 2), and intron (probe 3) sequences. A fourth probe was directed against the *td* intron (*td*i) (*6, 19*). Hybridization (37°C) and wash (46°C) conditions were as described (*19*). (**B**) DNA/RNA blots. Phenol-extracted RNA (R) was isolated from *E. coli* B that had been infected with the T-even phages for 12 minutes at 30°C and was spotted alongside the DNA (D). Conditions were as in (A) above, as were probes 1, 2, and 3. An additional *nrd*B intron probe (4) was used. (**C**) Map of the *nrd*B gene of T4 and hybridization probes. The 1762-bp *nrd*B gene is drawn to scale, with an intron of 598 bp (*9, 20*). Restriction enzyme sites are Hind III (H), Bgl II (B), Eco RI (E), and Kpn I (K). The bar above the map represents the intron ORF. Oligonucleotide probes (2 to 4) indicated by arrows are as described (*21*). While a nick-translated Hind III–Bgl II exon I probe hybridized to T2, T4, and T6 sequences the Eco RI–Kpn I intron fragment gave a signal with T4 only, corroborating the oligonucleotide probe data in (A) and (B).

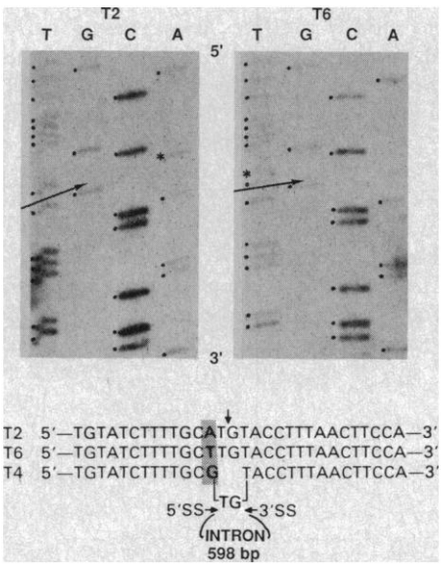

Fig. 3. Sequence analysis of the T2 and T6 *nrd*B genes. The sequences of T2 and T6 corresponding to the region in *nrd*B that contains an intron in T4 are shown. These sequences were determined with heat-denatured Aha III–restricted phage DNA templates by the dideoxy method, with an end-labeled exon II oligonucleotide primer (probe 1 in Fig. 2) (*21*) and reverse transcriptase. The absence of any intron sequence in the equivalent position to that in T4 *nrd*B is evident from inspection of the sequence (*9*). The contiguous sequence in T2 and T6 is shown below the sequencing gel, with the arrowhead indicating the position of the intron in T4. The splice sites (SS) are separated by a 598-bp intron in T4 (*20*). A variable nucleotide (asterisks on autoradiogram and shaded in sequence below) occurs in T2, T4, and T6 two bases upstream from the 5′ SS in T4. These mutations represent silent changes at the wobble position of an alanine codon. This is the only polymorphic site within 30 residues to either side of the intron insertion site in T4 *nrd*B. A relationship between this polymorphism and the recombination events that have resulted in the differences in *nrd*B intron occurrence in the T-even phages is an interesting possibility.

REFERENCES AND NOTES

1. T. R. Cech, *Cell* **44**, 207 (1986).
2. H. Nielsen and J. Engberg, *Nucleic Acids Res.* **13**, 7445 (1985).
3. A. Jacquier and B. Dujon, *Cell* **41**, 383 (1985); I. G. Macreadie, R. M. Scott, A. R. Zinn, R. A. Butow, *ibid.*, p. 395; L. Colleaux *et al.*, *ibid.* **44**, 521 (1986).
4. B. F. Lang, *EMBO J.* **3**, 2129 (1984).
5. F. Michel and D. Cummings, *Curr. Genet.* **10**, 69 (1985).
6. F. K. Chu, G. F. Maley, D. K. West, M. Belfort, F. Maley, *Cell* **45**, 157 (1986).
7. F. Michel and B. Dujon, *ibid.* **46**, 323 (1986).
8. J. M. Gott, D. A. Shub, M. Belfort, *ibid.* **47**, 81 (1986).
9. B.-M. Sjöberg *et al.*, *EMBO J.* **5**, 2031 (1986).
10. F. K. Chu, G. F. Maley, F. Maley, M. Belfort, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3049 (1984); K. Ehrenman *et al.*, *ibid.* **83**, 5875 (1986).
11. F. Chu *et al.*, *Fed. Proc.* **43**, 1772 (1984); J. Pedersen-Lane and M. Belfort, unpublished data; D. McPheeters and L. Gold, personal communication.
12. J.-S. Kim and N. Davidson, *Virology* **57**, 93 (1974).
13. G. V. Paddock and J. Abelson, *J. Biol. Chem.* **250**, 4207 (1975).
14. H. Gram and W. Rüger, *Mol. Gen. Genet.* **202**, 467 (1986).
15. T. R. Cech, *Int. Rev. Cytol.* **93**, 3 (1985); P. A. Sharp, *Cell* **42**, 397 (1985); W. Gilbert, *Nature (London)* **319**, 618 (1985).
16. J. E. Darnell and W. F. Doolittle, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1271 (1986).
17. M. Belfort *et al.*, in *New Perspectives on the Molecular Biology of RNA*, M. Inouye and B. Dudock, Eds. (Academic Press, New York, in press).
18. G. Garriga and A. M. Lambowitz, *Cell* **39**, 631 (1984).
19. D. H. Hall *et al.*, *ibid.* **48**, 63 (1987).
20. We have recently determined the *nrd*B splice junction with RNA from T4-infected cells and after self-splicing in vitro. These data, which are summarized in Fig. 3, predict an intron of 598 nucleotides (D. A. Shub, J. M. Gott, J. Pedersen-Lane, M. Belfort, in preparation).
21. Sequences of the *nrd*B probes are as follows: probe 1 (exon II, 66 to 87 nucleotides from 3′ splice site) =

5′-GGTGAAGCTGTTCATCACGTGC-3′; probe 2 (splice junction) = 5′-AGTTAAAGGTAC*ACG-CAAAAGATAC-3′ (the asterisk represents the splice junction); probe 3 (intron, noncoding, 73 to 95 nucleotides from 5′ splice site) = 5′-GGTGCAAGCAAAACCTTGGCTGC-3′; probe 4 (intron, ORF, 239 to 258 nucleotides from 5′ splice site) = 5′-GTCCCGTTCTTAACCATTTC-3′. The sequence of the *nrd*B gene has been described (*9*).

22. We wish to thank D. Shub and J. Gott for a critical reading of the manuscript, for many productive discussions, and for providing the T4 *nrd*B clones. We are also thankful to D. McPheeters and L. Gold for communicating unpublished results. Supported by grants from the National Science Foundation (DMB-8502961 and DMB-8505527) and the National Institutes of Health (GM 33314).

4 March 1987; accepted 20 May 1987

The Inversion of Sensory Processing by Feedback Pathways: A Model of Visual Cognitive Functions

E. HARTH, K. P. UNNIKRISHNAN, A. S. PANDYA

The mammalian visual system has a hierarchic structure with extensive reciprocal connections. A model is proposed in which the feedback pathways serve to modify afferent sensory stimuli in ways that enhance and complete sensory input patterns, suppress irrelevant features, and generate quasi-sensory patterns when afferent stimulation is weak or absent. Such inversion of sensory coding and feature extraction can be achieved by optimization processes in which scalar responses derived from high-level neural analyzers are used as cost functions to modify the filter properties of more peripheral sensory relays. An optimization algorithm, Alopex, which is used in the model, is readily implemented with known neural circuitry. The functioning of the system is investigated by computer simulations.

NUCLEI IN THE THALAMUS TRANSMIT information received from the senses to appropriate centers in the neocortex. These thalamic relays are also affected by reafferent neural pathways emanating from the neocortex and the brainstem reticular formation. The function of these feedbacks has long been a puzzle to neurophysiologists. In the visual system the cortex exhibits a variety of neural maps that preserve to some extent the retinotopic character of the stimulus: neighboring neurons represent neighboring points in sensory space. At the same time, feature-analyzing networks will tend to transform the initially retinotopic pattern into one in which codes are substituted for particular sensory features. Thus, at the cortical level, we know that single neurons express by their activity very specific features of the visual input and may be presumed to form part of feature analyzing systems (*1*). In the present model we propose a mechanism whereby central coded responses are able to recreate peripheral retinotopic activity. Both corticofugal fibers and brainstem afferents to the dorsal lateral geniculate nucleus (dLGN) play a role in this process.

In the mammalian visual system the dLGN is more than a passive relay between the retina and the visual cortex. The corticofugal fibers make up a large fraction of all inputs into the dLGN (*2*). In addition, fibers from centers in the brainstem and midbrain reticular formation are synaptically connected to neurons in the dLGN (*3*).

Figure 1 shows the connections between neurons in the dLGN, the perigeniculate nucleus (PGN), visual cortex, and afferents from the retina and reticular formation. Circles represent populations of neurons. The diagram shows the retinal afferents to geniculate relay cells (G) and interneurons

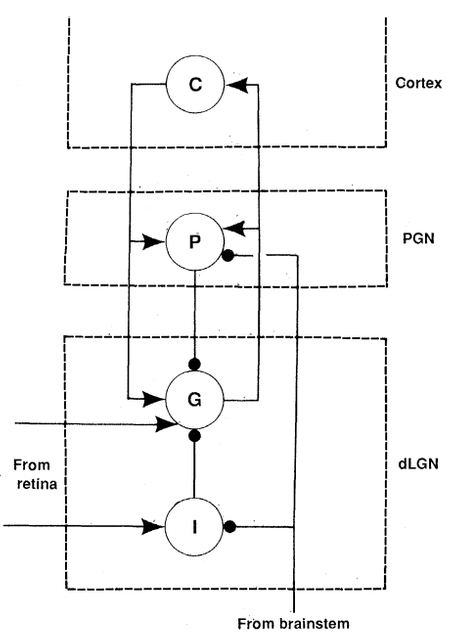

Fig. 1. Neural connectivity in the mammalian visual system. G, relay cells in dLGN; I, interneurons in dLGN; P, cells in PGN; C, cells in visual cortex. Open circles represent populations of neurons. Arrows represent excitatory synapses and filled circles represent inhibitory synapses.

(I). Fibers ascending from the dLGN to the cortex make collateral connections with PGN cells (P), and corticofugal fibers synapse with PGN cells on their way back to the dLGN (*4*). PGN cells are inhibitory on relay cells in the dLGN and receive diffuse inputs from the brainstem reticular formation. We have taken these inputs to be inhibitory (*2*). Unlike the corticofugal fibers, which are retinotopically arranged, afferents from the reticular formation, at least those emanating from the locus coeruleus, appear to be diffusely distributed over the dLGN (*5*).

Visual information is evidently modified on its way to the cortex, and the modifications carry with them information derived from higher level cortical analyzers and the presumably more global information from the reticular core.

Visual centers in the cortex also exhibit reciprocal innervation (*6*), and are affected by reafferents from the reticular formation (*7*). The mammalian visual pathway, after the retina, thus appears as a succession of mutually interacting centers, which are also subjected to the global influence of the reticular core.

Figure 2A illustrates the mechanism by which inverse sensory processing may be achieved through feedback. This was discussed in earlier versions of the model (*8*). Here, an array $\{x\}$ is incident on a sensory relay (a) that passes the information in modified form $\{y\}$ to a set of analyzers (b). The responses of these analyzers form a new array $\{r\}$ whose components reflect the presence of particular features in $\{y\}$. Responses are calculated as inner products between $\{y\}$ and static templates, and are multiplied by coefficients that define the sensitivities of the analyzers (*9*). All responses are summed in (s), producing a scalar quantity ρ. The summation is nonlinear, so that ρ is sensitive to the emergence of a single component of $\{r\}$, rather than to a superposition of many weaker responses. This is accomplished, for example, by adding the responses raised to a power greater than one.

Modification of $\{x\}$ proceeds by an iterative optimization process. Maximizing the global feedback ρ will have the following consequences:

1) If $\{x\}$ has some initial resemblance to one of the features defined by the analyzers and if the sensitivities of the analyzers are all the same, the pattern should converge on that feature. Missing parts of the pattern will be completed and extraneous features suppressed.

E. Harth and K. P. Unnikrishnan, Department of Physics, Syracuse University, Syracuse, NY 13244.
A. S. Pandya, School of Computer and Information Science, Syracuse University, Syracuse, NY 13244.