38. J. Sambrook, E. F. Fritsch, T. Maniatis, in *Molecular Cloning: A Laboratory Manual*, C. Nolan, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989).
39. Hybridization was allowed to proceed for 7 hours at 55°C, a temperature calculated to maximize the stability of DNA-RNA hybrids. The samples were digested by nuclease S1 (5000 U/ml) for 1 hour at 37°C, stopped as described in (*38*), precipitated, separated by electrophoresis on a 5% polyacrylamide–7 M urea gel, and autoradiographed.
40. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
41. D. A. Hager and R. R. Burgess, *Anal. Biochem.* **109**, 76 (1980).
42. S. Anderson *et al.*, *Nature* **290**, 457 (1981).
43. D. D. Chang and D. A. Clayton, *EMBO J.* **6**, 409 (1987).
44. L. Wen *et al.*, *Nucleic Acids Res.* **17**, 1197 (1989).
45. B. T. Pentecost *et al.*, *ibid.* **13**, 4871 (1985).
46. M.A.P. thanks G. A. M. Breen for purification of protein and advice and support during sabbatical leave in this laboratory. We thank S. J. Elledge for the human cDNA library and D. L. Brutlag and H.-M. Jantzen for discussions of sequence alignments. We acknowledge the Protein and Nucleic Acid Facility, Beckman Center, for the synthetic oligonucleotides and amino acid analysis, and we thank R. P. Fisher and J. N. Doda for assistance; J. F. Hess, J. L. Bennett, L. L. Stohl, C. Szymkowiak, J. Poulton, and T. Lisowsky for reagents and advice; M. E. Schmitt and J. L. Bennett for comments and K. Shaw for help in preparation of the manuscript. M.A.P. is a Medical Scientist Training Program Trainee of the National Institute of General Medical Sciences (GM07365-15). Supported by grants from the National Institute of General Medical Sciences (GM33088-20) and from the American Cancer Society, Inc. (NP-9P).

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Retronphage φR73: An *E. coli* Phage That Contains a Retroelement and Integrates into a tRNA Gene

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Some strains of *Escherichia coli* contain retroelements (retrons) that encode genes for reverse transcriptase and branched, multicopy, single-stranded DNA (msDNA) linked to RNA. However, the origin of retrons is unknown. A P4-like cryptic prophage was found that contains a retroelement (retron Ec73) for msDNA-Ec73 in an *E. coli* clinical strain. The entire genome of this prophage, named φR73, is 12.7 kilobase pairs and is flanked by 29–base pair direct repeats derived from the 3′ end of the selenocystyl transfer RNA gene (*selC*). P2 bacteriophage caused excision of the φR73 prophage and acted as a helper to package φR73 DNA into an infectious virion. The newly formed φR73 closely resembled P4 as a virion and in its lytic growth. Retronphage φR73 lysogenized a new host strain, reintegrating its genome into the *selC* gene of the host chromosome and enabling the newly formed lysogens to produce msDNA-Ec73. Hence, retron Ec73 can be transferred intercellularly as part of the genome of a helper-dependent retronphage.

A SATELLITE DNA, CALLED MSDNA, has been discovered in myxobacteria and some clinical strains of *E. coli* (*1*). These molecules consist of single-stranded DNA that is branched out from an internal guanosine residue by a 2′,5′ phosphodiester linkage. Reverse transcriptase (RT) is required for the synthesis of msDNA. We found an msDNA, called msDNA-Ec73, in an *E. coli* clinical strain (*2*). The msDNA-Ec73 is produced by a 2.4-kb retron (retron Ec73) that consists of the genes for DNA-linked RNA and msDNA, an open reading frame (ORF) that encodes a protein of unknown function (ORF 316), and the gene for an RT. Retron Ec73 is part of a 12.7-kb fragment of foreign DNA flanked by 29-bp direct repeats that was integrated into the 3′ end of the gene for selenocystyl tRNA (*selC*) at 82 min on the *E. coli* chromosome (*2*).

Except for the presence of the retron region, the 12.7-kb segment resembles the genome of satellite coliphage P4. Its ORFs show sequence similarity to genes and ORFs of P4 and have the same arrangement as on the P4 genome. This suggests that retron Ec73 exists in the genome of a prophage. This prophage, called a φR73, differs from P4 prophage in its location on the *E. coli* chromosome (P4 is integrated at 97 min) and in the flanking direct repeats (a different 20-bp sequence than for P4) (*3*).

We tested whether the φR73 prophage can be induced to generate an infectious retronphage. This would allow transfer of retron Ec73 by integration of the prophage into the *E. coli* chromosome. Phage P4 requires a helper genome, such as that of coliphage P2, to provide the late gene functions for its lytic growth (*3*). Because the φR73 genome resembles that of P4, we tested whether P2 could serve as helper for excision of φR73. For P2 to be a helper, φR73 DNA must contain a *cos* site, which allows P2 to package φR73 DNA into a phage head. Indeed, a segment of the φR73 DNA contains a 19-bp sequence that is similar to the consensus sequence of P2, P4, and phage 186 that exists between the sites that produce the ends of packaged DNA (*3*).

The plasmid pCl-23a (*4*) is about 18 kb in size; thus its dimer (36 kb) is only slightly larger than the P2 genome (33 kb). If the φR73 *cos* site is P2-compatible, P2 should be able to package pCl-23a as a dimer. To test this possibility, we infected *E. coli* C1a cells that carried pCl-23a with P2. We found that we could transduce pCl-23a into another *E. coli* strain (C366) that carries a P2 prophage (*5*). The transductant produced a low amount of phage that formed P4-like plaques with indicator strains lysogenic for P2. Phage isolated from one such plaque served as a source for the φR73 wild-type phage. That φR73, like P4, depended on a helper phage for its lytic growth was evident from its inability to form plaques on lawns of *E. coli* strains that lacked a P2 prophage.

Electron microscopic examination of a negatively stained preparation of retronphage φR73 indicated that its morphology was indistinguishable from that of P4. Both phages have icosahedral heads attached to tails with a contractile sheath (Fig. 1). We

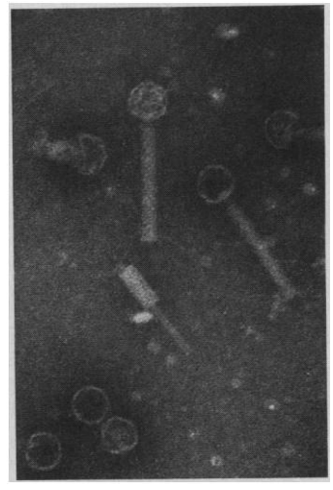

Fig. 1. Electron micrograph of P4-like retronphage φR73. Phage from φR73 lysate was negatively stained with phosphotungstate (*10*) and viewed under a Hitachi H-7000 electron microscope. The tail of the φR73 virion should be the same as that of its helper, phage P2, and may therefore serve as size reference (tail length, 135 nm).

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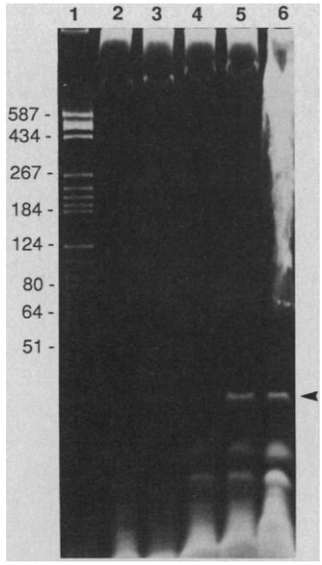

Fig. 2. The msDNA production of strains C366, C367, C1a, C368, and Cl-23. DNAs were isolated as described (*11*) from stationary growing cells (14 ml). After treatment with ribonuclease A, samples were applied to 10% polyacrylamide gels. DNA was visualized by staining with ethidium bromide. Lane 1, pBR322 DNA digested with Hae III as a molecular size standard; lane 2, C366; lane 3, C367; lane 4, C1a; lane 5, C368; and lane 6, Cl-23. An arrowhead indicates the position of msDNA-Ec73. Numbers at left are sizes of the DNA fragments (in base pairs).

determined the relative dimensions for a few ϕR73 particles. The width-to-length ratio for the tail (0.13) corresponded to that found for P2 and P4. The ratio of head diameter to tail length (0.33) was characteristic of P4 and not of P2 (0.44) (*3*), indicating that the ϕR73 genome provided a P4-like *sid* function.

We further characterized ϕR73 by infections of L broth cultures of C295 (C1a lysogenic for P2 wild type) as was done for P4 (*6*). In this host, ϕR73 exhibited a latent period of ~55 min (7), slightly shorter than the 60-min latent period characteristic forP4. The yield of ϕR73 was five plaque-forming units per cell, much lower than that of P4 (~100). Addition of divalent cations to the medium (2 mM $MgCl_2$ or 5 mM $CaCl_2$) increased the yield 20-fold (for $MgCl_2$, mean = 97). This finding suggests that the ϕR73 virion needs to be stabilized by cations, perhaps because the size of the ϕR73 DNA (12.7 kb) exceeds that of P4 DNA (11.6 kb) and therefore is packed more tightly in a phage head.

Like P4, ϕR73 trans-activated its helper phage, enabling it to grow in C315, a C1a derivative lysogenic for a P2 prophage that is replication-deficient, P2Aam 127 (*6*). However, for this host the ϕR73 yield (mean = 16 plaque-forming units per cell, with 2 mM $MgCl_2$) was lower than that for P4 (mean = 166) (*6*). This was not surprising, as the trans-activation gene δ of P4 differs from its probable ϕR73 counterpart (*2*). Retronphage ϕR73 efficiently complemented a gene α–defective P4 mutant, *am*1 (*8*). Our findings that ϕR73 displays functions in its lytic growth cycle equivalent to those of the products of the P4 genes α, ϵ, *sid*, δ, and *psu* suggests that the corresponding ϕR73 gene products fulfill the same functions for ϕR73.

Retronphage ϕR73 forms turbid plaques and lysogenizes its host. We obtained ϕR73 lysogens by infecting C1a cells and C366 cells, the P2 lysogen, with ϕR73. C368, the ϕR73 lysogen of C1a, produced ϕR73 phage when infected with P2. The ϕR73-P2 double lysogen, C367, released retronphage ϕR73 at a rate similar to that of P4 release from cells that carry P2 and P4 prophages. The fact that ϕR73 can lysogenize its host implies that it has a functional *int* gene.

Using a DNA fragment that contained the genes for retron Ec73 as a probe for plaque hybridization, we showed that the newly formed phage particles contained retron Ec73 in their genome. All of the ϕR73 plaques hybridized with the probe, although no plaque produced by P4 hybridized with the probe. Furthermore, newly constructed ϕR73 lysogens produced msDNA (Fig. 2). Strains C366 (P2 lysogen) and C1a (P2 nonlysogen) did not produce msDNA (lanes 2 and 4, respectively). The size of msDNA produced by these strains after lysogenization with ϕR73 (lanes 3 and 5) was identical to that of msDNA-Ec73 produced by the original clinical strain Cl-23 (lane 6).

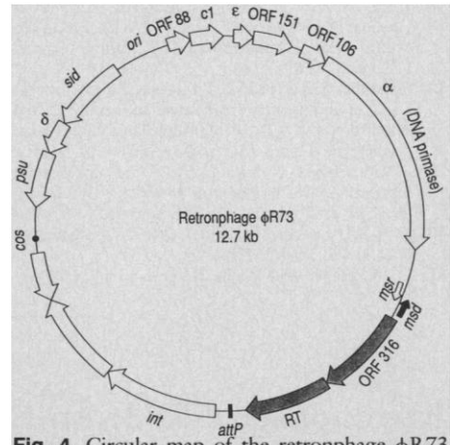

Fig. 4. Circular map of the retronphage ϕR73 genome. Gene assignments are from (*2*). ORF316 and Ec73-RT are shaded. The directions of ORFs are indicated by arrows. The arrow sizes are proportional to the sizes of genes.

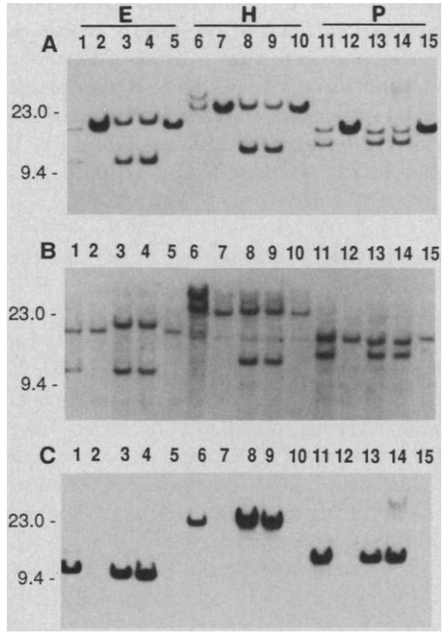

Fig. 3. Southern blot hybridization analysis of the integration sites of retronphage ϕR73. Chromosomal DNA digested with Eco RI, Hind III, and Pst I (indicated by E, H, and P on bars, respectively). Digests were resolved on a 0.7% agarose gel. Lanes 1, 6, and 11 are Cl-23; lanes 2, 7, and 12 are C366; lanes 3, 8, and 13 are C367; lanes 4, 9, and 14 are C368; and lanes 5, 10, and 15 are C1a. (**A**) The ^{32}P-labeled 1.5-kb Eco RI fragment from K-12 DNA containing the *selC* gene (*2*) was used as a probe. (**B**) The 29-nucleotide probe corresponding to Cl-23L [ACTCCTGTGATCTTCCGCCAAAATTCCTC (*2*)] was labeled with T4 polynucleotide kinase and [γ-^{32}P]adenosine triphosphate and used as a probe. (**C**) The nick-translated 1.6-kb Sal I–Bgl II fragment that encompassed the retron Ec73 was used as a probe. Markers at left indicate sizes in kilobases.

In order to determine the site of integration of retronphage ϕR73 in strains C367 and C368, we performed Southern (DNA) blot hybridization with a DNA fragment derived from the *E. coli* K-12 chromosome as a probe. This fragment contained the site where proretronphage ϕR73 integrates in strain Cl-23 (*2*). In both of the strains C366 and C1a, a single DNA fragment was detected with the *selC*-containing probe (Fig. 3A). However, when these strains were lysogenized with ϕR73, two new fragments were identified that corresponded to those in the original clinical strain Cl-23. Thus, ϕR73 integrated into the same site on the *E. coli* chromosome as that observed in Cl-23. To demonstrate the site-specific integration of the prophage at the *attB* site in the *selC* gene, we carried out Southern blot hybridization with a 29-nucleotide probe that had the same sequence as *attB* (Fig. 3B). DNA fragments identical to those shown in Fig. 3A were generated, indicating that one *attB* sequence existed before lysogeny, whereas the *attB* sequence was duplicated in lysogenized cells. Using a fragment from retron Ec73 as a probe, we confirmed that ϕR73-

lysogenic strains contained retron Ec73 (Fig. 3C). Only one fragment was detected in lysogenized strains Cl-23, C367, and C368.

The 12.7-kb genome of retronphage ϕR73 can be shown as a circular map (Fig. 4) (*2*). The α gene is the only P4 gene that is essential for lytic growth (*3*), and retronphage ϕR73 can complement the α gene–defective P4 mutant. DNA synthesis of the retronphage ϕR73 genome could be primed by α primase at ORI and at two other sites, one at the 3′ end of *msd* and the other at the *attB* site. DNA synthesis initiating at *msd* would use msDNA as a primer and RT for DNA elongation. DNA synthesis initiating at *attB* would use selenocystyl tRNA as a primer and RT for DNA elongation. In both cases, RNA transcripts would be used as templates. The *E. coli* RT from retron Ec67 can use various primers and templates besides its own endogenous primer and template (*9*). It has not yet been determined whether such DNA synthesis participates in the life-cycle of retronphage ϕR73.

REFERENCES AND NOTES

1. M. Inouye and S. Inouye, *Trends Biochem. Sci.* **16**, 18 (1991).
2. J. Sun, M. Inouye, S. Inouye, *J. Bacteriol.*, in press.
3. L. E. Bertani and E. W. Six, in *The Bacteriophages*, R. Calendar, Ed. (Plenum, New York, 1988), vol. 2, p. 73.
4. Plasmid pCl-23a contains the 14.4-kb Bgl II–Eco RI fragment containing the entire ϕR73 that was cloned in pBR322 (*2*).
5. C366 is a P2*int*1 lysogen of Cl792 (*8*); the *int*1 mutation inactivates the P2 *int* gene and thereby blocks P2 prophage excision and coproduction of P2 after infection with P4-like phage.
6. E. W. Six, *Virology* **67**, 249 (1975).
7. We measured the latent period of ϕR73 infection by monitoring the optical density of the culture after infection. When infected cells lyse, the optical density drops, and the time required for the lysis is defined as the latent period.
8. W. Gibbs, R. Goldstein, R. Weiner, B. Lindquist, R. Calendar, *Virology* **53**, 24 (1973).
9. B. C. Lampson, M. Viswanathan, M. Inouye, S. Inouye, *J. Biol. Chem.* **265**, 8490 (1990).
10. J. T. Walker and D. H. Walker, *J. Virol.* **45**, 1118 (1983).
11. M. C. Birnboim and J. Dolly, *Nucleic Acids Res.* **7**, 1513 (1979).

12. Electron microscopy services were provided by the University of Iowa Central Electron Microscopy Research Faculty. We thank D. Cue for his help with the electron microscopy. Supported by NIH grant GM44012 (M.I. and S.I.) and AI04043 (E.W.S.)

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Spectral Tuning of Pigments Underlying Red-Green Color Vision

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Variations in the absorption spectra of cone photopigments over the spectral range of about 530 to 562 nanometers are a principal cause of individual differences in human color vision and of differences in color vision within and across other primates. To study the molecular basis of these variations, nucleotide sequences were determined for eight primate photopigment genes. The spectral peaks of the pigments specified by these genes spanned the range from 530 to 562 nanometers. Comparisons of the deduced amino acid sequences of these eight pigments suggest that three amino acid substitutions produce the approximately 30-nanometer difference in spectral peaks of the pigments underlying human red-green color vision, and red shifts of specific magnitudes are produced by replacement of nonpolar with hydroxyl-bearing amino acids at each of the three critical positions.

HUMAN COLOR VISION ENCOMpasses a range of individual variations, including the classically defined categories of anomalous and defective color vision (*1*) and a range of more subtle variations among individuals whose color vision is considered to be normal (*2, 3*). One of the principal causes of these color vision differences is variation in the spectral positioning of the cone photopigments. In turn, the spectral tuning of visual pigments is thought to be governed by interactions between the 11-*cis* retinal chromophore and the apoprotein, opsin (*4–6*). An essential step toward understanding the molecular basis of color blindness and other color vision variations is, thus, to determine the structural variations among opsins that govern the spectral absorption properties of the visual pigments.

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We have identified amino acid substitutions likely to be involved in tuning the absorption spectra of naturally occurring visual pigments that underlie red-green color vision by comparing the deduced amino acid sequences of eight cone pigments with absorption peaks between 530 and 562 nm. These pigments are a middle wavelength–sensitive (MWS) pigment from a human protanope (7), a long-wavelength–sensitive (LWS) pigment from a human deuteranope, and three pigments from each of two species of South American monkey [squirrel monkeys (*Saimiri sciureus*) and saddle-backed tamarins (*Saguinus fuscicollis*)]. The genes encoding all of these visual pigments reside on the X chromosome (*8– 10*). The monkeys have a single X-chromosome locus with three allelic genes.

Spectral sensitivities of the pigments (Fig. 1) were determined by analysis of a retinal gross potential, the electroretinogram (ERG), recorded from the eye of each subject. To measure relative sensitivity, we adjusted the quantal intensity of a rapidly flickering monochromatic light to produce a response that matched in amplitude the response to an interleaved flickering reference light. Repetition of this procedure for many test wavelengths yielded a curve that characterizes the absorption spectra in dichromatic subjects of cone pigments that are sensitive to middle to long wavelengths (*7, 8, 11*).

Of the six exons composing X-linked visual pigment genes, only exons 2 to 5 specify membrane-spanning regions of opsin (*10*), and thus only these exons are likely to be linked to spectral tuning of the photopigments. Gene segments corresponding to these exons were amplified in genomic DNA from each animal with the polymerase chain reaction (PCR), and the nucleotide sequences were determined (*12*). No polymorphisms were found in the pigment gene sequences from individual monkeys, confirming that each has a single X-encoded pigment (*8, 9*). This was also true of the human deuteranope. The deduced amino acid sequences of the monkey pigments are from 96% to >98% identical to the human LWS pigment.

Which amino acid substitutions might account for the spectral differences among these? The human protanope and deuteranope pigments differ by 11 amino acid substitutions; seven of these are nonhomologous substitutions (Fig. 2A). A subset of these seven most likely accounts for the approximately 30-nm difference in the absorption maxima of the photopigments. Three of the monkey pigments have spectral peaks (556, 547, and 541 nm) that are clearly intermediate to those of the human pigments. Three pairwise comparisons can be made among the monkey pigments in which each pair differs at only one of the seven positions where nonhomologous substitutions occur between the human pigments. The 556-nm tamarin pigment differs