transcription above a basal level and compete with GAL4/VP16 for transcriptional factors. This model is consistent with recent evidence indicating different conformational states of Leu3p and a relation between these states and transcriptional effectiveness (*11*).

Steroid hormone receptors and the yeast ACE1 metallothionein regulator are two examples of eukaryotic transcriptional activators that require small molecule cofactors (*13*). In these examples the ligand affects DNA binding; the effect on activation is indirect. For Leu3p, DNA binding is constitutive: the ligand affects activation directly. In addition to serving as a model system for the study of metabolite-dependent eukaryotic gene regulation in vitro, Leu3p–α-IPM activation is expected to facilitate the analysis of interactions between an activator and the components of the RNA polymerase II transcription complex.

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14. We constructed the UAS−, basal template, pJJ469, by subcloning the CYC1 TATA and G-less cassette sequences from pGAL4CG− as an Sma I fragment into the Sma I site of pUC18 (*6*). Templates with one (pJJ534) or two (pJJ482) UAS_{LEU} sites were derivatives of pJJ460 (*6*). The UAS_{LEU} sequence used in the templates was identical to that used in the band shift assays (*3, 4*).
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4 May 1992; accepted 19 August 1992

Bacteriophage λ*PaPa*: Not the Mother of All λ Phages

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The common laboratory strain of bacteriophage λ—λ wild type or λ*PaPa*—carries a frameshift mutation relative to Ur-λ, the original isolate. The Ur-λ virions have thin, jointed tail fibers that are absent from λ wild type. Two novel proteins of Ur-λ constitute the fibers: the product of *stf*, the gene that is disrupted in λ wild type by the frameshift mutation, and the product of gene *tfa*, a protein that is implicated in facilitating tail fiber assembly. Relative to λ wild type, Ur-λ has expanded receptor specificity and adsorbs to *Escherichia coli* cells more rapidly.

The tail fibers of the double-stranded DNA bacteriophages are the organelles through which the virus contacts its host in the first steps of the infection process. By forming specific contacts with receptor molecules on the surface of the bacterial cell, the tail fibers provide a primary determinant of the host range of the bacteriophage that carries them. Mutations in tail fiber genes can lead to changes in receptor specificity that are thought to allow the phage to adapt to populations of bacteria with different or mutationally altered surface receptors. Some tail fibers show a remarkable plasticity in the range of receptor specificities they can access through small mutational changes (*1*).

A different evolutionary plasticity in the tail fiber structure in phage populations is suggested by recent comparisons of DNA sequences of phage tail fiber genes (*2, 3*). These studies revealed that tail fiber genes from different phages that were thought to be unrelated share stretches of sequence similarity. From these observations, there appears to be rather widespread and promiscuous sharing of tail fiber gene parts, and presumably receptor specificities, among populations of phages. Among the surprising observations from these sequence comparisons was that the tail fiber gene (gene *H*) of *Escherichia coli* phage P2 shares a sequence similarity with *E. coli* phage λ that extends across two adjacent open reading frames in the λ sequence. These open reading frames are located within the central "nonessential" region of the λ genome, near the end of the late gene operon. The P2 and λ sequences align with ~78% local identity if a single base gap is introduced into the λ sequence; if a base were to be inserted into the λ DNA at the position of the gap in the sequence alignment, the two open reading frames would fuse into one large open reading frame. These observations led Haggård-Ljungquist *et al.* (*2*) to suggest that there may exist versions of λ in which this hypothetical gene is intact and that it encodes a tail fiber not present on the laboratory version of λ. It had been recognized earlier that a region near the end of this hypothetical gene is similar in sequence to the corresponding part of the phage T4 distal half fiber gene, gene *37* (*4*), and that this part of the λ sequence (together with the downstream gene) can be substituted into T4 to produce a T4 phage with altered host range (*5*).

Phage λ was first reported in 1951 (*6*) as a phage derived from a prophage residing in *E. coli* strain K12, itself isolated from a clinical specimen in California in 1922 (*7*). However, the version of λ studied in laboratories around the world for most of the past 40 years is not identical to the version originally isolated from *E. coli* K12. Instead, nearly all strains of λ in current use are

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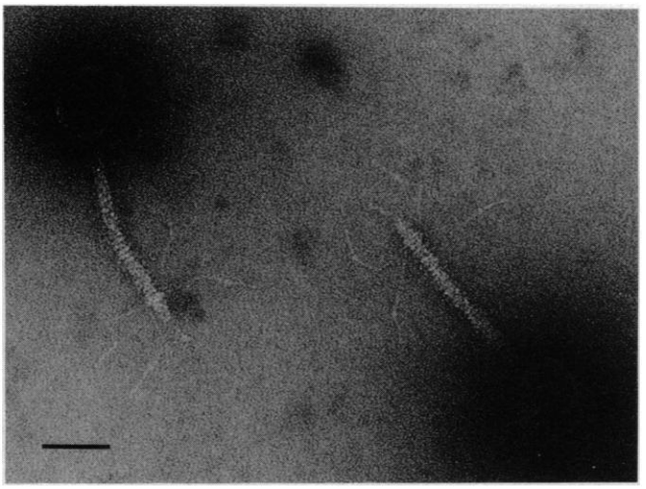

Fig. 1. Virions of Ur-λ negatively stained with uranyl formate on unsupported carbon film (*29*). Phage were produced by ultraviolet induction of *E. coli* strain K12(λ) (*30*). Phage were collected by centrifugation of the crude lysate, resuspended, purified by centrifugation on a CsCl gradient, and dialyzed into 20 mM tris-HCl, pH 7.5, 2 mM $MgCl_2$ prior to preparation for electron microscopy. Grids were examined in a Zeiss EM902 electron microscope at an accelerating voltage of 80 kV. The scale bar represents 50 nM.

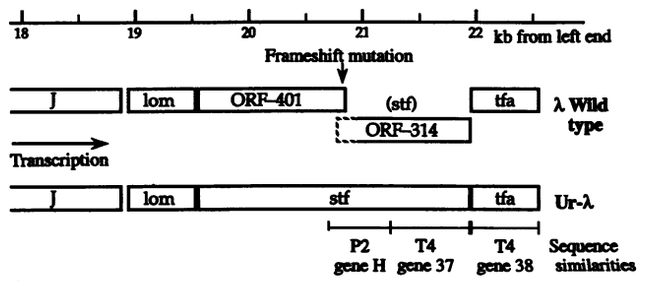

Fig. 2. Physical map of the λ tail fiber region, comparing λ wild type (λ*PaPa*) and Ur-λ. The gene name *stf* (side tail fiber) was originally applied by Montag *et al.* (*5*) to ORF-314. Since ORF-314 now appears to be a gene fragment caused by the indicated frameshift mutation, we propose [in agreement with the usage of Haggård-Ljungquist *et al.* (*2*)] to transfer the designation *stf* to the intact gene as found in Ur-λ. The arrow indicates the location of the single base pair deficiency in λ wild type relative to Ur-λ. *Stf* encodes the larger of the two new tail fiber proteins of Ur-λ; *tfa* [tail fiber assembly (*18*)] encodes the smaller protein. Gene *J* encodes the "traditional" λ tail fiber located at the tip of the tail (*10*); *lom* encodes an outer membrane protein of unknown function (*31*).

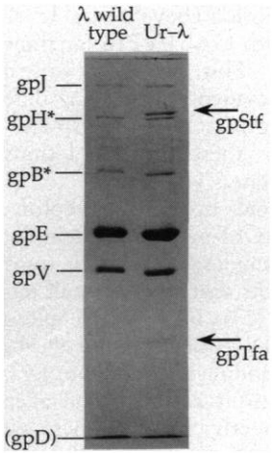

Fig. 3. Sodium dodecyl sulfate–polyacrylamide gel of Ur-λ (right lane) and λ wild-type (left lane) virions. The new bands in Ur-λ are indicated with arrows. Approximate molecular sizes of the previously identified bands are gpJ, 124 kD; gpH*, 78 kD; gpB*, 58 kD; gpE, 38 kD; gpV, 26 kD; and gpD, 12 kD.

descendants of a recombinant, λ*PaPa*, derived (*8*) from a cross between strains of λ then in use in *Pa*sadena (Caltech) and in *Pa*ris (Institut Pasteur) (*9*). The λ*PaPa* strain was chosen to have desirable features of both parents namely certain immunity properties of the Paris strain and a medium plaque size phenotype of the Pasadena strain. The λ*PaPa* strain is also referred to as λ wild type.

We now report studies on a strain of λ obtained by ultraviolet light induction of a version of *E. coli* K12(λ) thought to be close if not identical to the strain from which Lederberg first isolated λ (*6*). We call this strain Ur-λ to distinguish it from λ wild type.

In addition to the familiar icosahedral head and flexible noncontractile tail, Ur-λ has thin, angular fibers that appear to extend from the sides of the conical tip of the tail (Fig. 1). The fibers lie in various attitudes on the grid, but most appear to consist of three straight segments connected by flexible joints. The segment proximal to the tail is approximately 35 nm long and has a thin, uniform diameter. It joins to the middle segment, which is thicker and more variable in diameter with a length of 20 to 25 nm and which joins in turn to the distal segment, which is approximately 25 nm long with a thin, uniform diameter. The number of fibers clearly displayed varies among different virions, with four fibers being visible on many individual virions but larger numbers being visible only rarely. Our results appear to be consistent with six fibers per virion, a number that would match the rotational symmetry of the tail as well as the number of fibers on many other phages. More difficult to see, but visible in some cases, is the short, thin fiber coming from the very tip of the tail. This is the traditional λ tail fiber encoded by gene *J* (*10*).

The sequence comparisons of Haggård-Ljungquist *et al.* (*2*) suggested that λ wild type carries a frameshift mutation in the middle of a tail fiber gene. The novel tail fibers visible on Ur-λ could plausibly contain the product of that gene if it is intact in Ur-λ. We determined the DNA sequence of Ur-λ across the region of the putative mutation. As predicted, the Ur-λ sequence differs from the λ wild-type sequence by having an extra base pair (an additional cytosine in the coding strand inserted into the run of three cytosines at positions 20833 to 20835) relative to the λ wild-type genome sequence of Sanger *et al.* (*11*). This change should restore to a single gene the two open reading frames in the λ wild-type sequence that show sequence similarity to the phage P2 tail fiber gene (Fig. 2). The intact λ gene should encode a 774–amino acid protein.

An SDS-polyacrylamide gel of the virion proteins of purified Ur-λ and λ wild type showed that the two patterns are identical except for the presence of two additional bands in the Ur-λ pattern at positions corresponding to apparent masses of ~80 and ~20 kD (Fig. 3). Amino-terminal amino acid sequences (*12*) of proteins extracted from these two bands are AVEISGVLKD (*13*) for the ~80-kD protein and AFRMSEQP?TIKIY for the ~20-kD protein. This identifies them, respectively, as the product of the new tail fiber gene, which we call *stf*, with a predicted molecular mass of 77,481 daltons, and the product of the next gene downstream, *tfa*, with a predicted molecular mass of 21,588 daltons. Quantitation of gel bands such as those in Fig. 3 indicates that there are about 10 to 12 copies per virion of each of these two proteins (*14*).

If the fibers on Ur-λ are functional we might expect them to affect the way this phage adsorbs to its host. The rates of adsorption to an *E. coli* K12 strain for Ur-λ and λ wild type were examined (Fig. 4). The bacteria were grown either in the presence of 0.4% maltose or 0.4% glucose. Maltose induces expression of *lamB*, which encodes the receptor (*15*) recognized by the

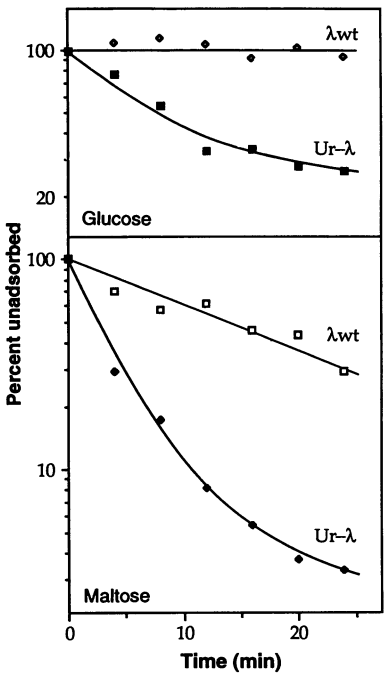

Fig. 4. Adsorption kinetics of Ur-λ and λ wild type on *E. coli* strain Ymel. Cells were subcultured and grown to log phase in tryptone yeast extract broth supplemented with 2 mM $MgCl_2$ and either 0.4% maltose or 0.4% glucose. Phage at a concentration of 5×10^4 pfu/ml were diluted (10 volumes) into cells at 2.5×10^7 cells per milliliter and incubated at 37°C. At the indicated times a portion was removed, the cells were sedimented, and the supernatant was analyzed for phage activity.

gpJ tail fiber protein present on both Ur-λ and λ wild type, and glucose represses *lamB* expression. In the glucose-grown cells, λ wild type did not adsorb detectably, presumably reflecting a deficiency of LamB receptor on these cells, but Ur-λ adsorbed at an appreciable rate. In the maltose-grown cells, λ wild type now adsorbed at a measurable rate. Ur-λ adsorbs to these cells substantially faster than λ wild type. These results may imply that the side tail fibers of Ur-λ endow it with the ability to adsorb through a non-LamB receptor that is present on both glucose- and maltose-grown cells. Although we have not identified this receptor explicitly, the experiments of Montag *et al.* (*5*), in which *tfa* and part of *stf* were substituted for the homologous regions of the phage T4 tail fibers, suggest that the receptor is the outer membrane protein OmpC.

The identification of the two new proteins in Ur-λ as components of the side tail fibers rests on two pieces of evidence. First, the presence of the fibers correlates with the presence of these proteins in the virion in amounts appropriate to constitute the fibers. Second, the sequences of both proteins provide support for their relation to tail fiber proteins of other phages. A central portion of the large protein gpStf, as mentioned earlier, is similar to a portion of the phage P2 gene *H* tail fiber protein. The COOH-terminal portion of gpStf is similar to the corresponding portion of the phage T4 gene 37 protein, a dimer of which constitutes most of the distal half tail fiber of that phage (*16*) (Fig. 2). The smaller λ protein, gpTfa, is 36% identical in amino acid sequence to the gene *38* protein of T4 (*17*), and the λ *tfa* gene can in fact complement a T4 38^- mutant (*18*).

In T4, the gene *38* protein is implicated in promoting correct assembly of the fiber protein, gp37, but it is not itself found as a component of the mature virion (*19*). In the related phage T2, the corresponding gene *38* protein (which is unrelated in sequence to T4 gp38 or λ gpTfa) is not required for assembly of the gp37 tail fiber protein, but it is a component of the mature virion. It is located at the tip of the fiber and is the protein that contacts the host receptor (*20*). Thus, the λ protein appears to share sequence similarity and assembly-promoting activity with the T4 protein but assembly behavior with the T2 protein. This comparison suggests that the assembly behavior of gpTfa and its T–even phage homologs (that is, whether or not they remain associated with the mature structure) may be an additional feature, together with segments of tail fiber sequence, that can be assorted combinatorially during phage evolution to produce tail fibers with new properties.

There has been speculation but little direct evidence about how the T4 gp38 protein promotes assembly of the gp37 subunit into the dimer that forms the distal half tail fiber. Whatever the mechanism, the observation that λ gpTfa can substitute for T4 gp38 implies that the λ protein shares that mechanism. We suggest that, in the case of the λ tail fiber, which unlike the T4 fiber retains the assembly-promoting protein, the assembly-promoting complex may be preserved, and studies of its structure may give clues about how one protein can direct another protein's assembly.

The two tail fiber genes responsible for the Ur-λ side tail fibers, *stf* and *tfa*, lie in the central portion of the λ genome. Because deletion of this region has no detectable phenotypic effect, it is designated as nonessential even though it encodes proteins from both early and late transcripts (*21*). (This is the region that is deleted in most λ-based cloning vectors to make space for cloned DNA.) Our data make it clear at least for the *stf* and *tfa* genes that the reason their deletion from λ wild type has no phenotypic effect is that tail fiber production is already prevented in λ wild type by the frameshift mutation in *stf*. Identification of *stf* and *tfa* as genes with clear roles in λ tail production extends the tail genes of λ halfway through the nonessential region to the very end of the late operon.

Even though the so-called nonessential genes *stf* and *tfa* are essential for side tail fiber formation, they are nonetheless nonessential in the sense that a phage without the side tail fibers grows perfectly well, albeit with altered adsorption kinetics. Another phage with side tail fibers like those of Ur-λ is coliphage T5, which like λ can still make plaques with good efficiency if its side tail fibers are removed by mutation (*22*). The question arises whether presence and absence of side tail fibers might be naturally occurring alternative conditions of phages like λ and T5. Genetic switching between alternate tail fiber arrangements on a rapid time scale is documented for phages Mu (*23*) and P1 (*24*). The absence of tail fibers in λ wild type apparently arose in the laboratory as the result of the manipulations that led to λ*PaPa*, so in this case the lack of tail fibers does not necessarily represent a naturally occurring condition. However, other independently isolated lambdoid phages (for example, φ80 and HK97) appear, by electron microscopy, to lack side tail fibers (*25*) and might therefore represent natural isolates of phages in the side fiber off condition. In at least one of these cases, HK97, there are some sequence data suggesting that, despite the absence of side tail fibers, the phage does have genes homologous to *stf* and *tfa* of λ (*26*).

Like the λ's studied in the early 1950's (*27*), our Ur-λ makes very small (~0.5 mm) plaques. It has been suggested that the small plaque phenotype may correlate with presence of the side tail fibers (*2*). We have confirmed this by showing that, if λ*cI857Sam7* (a λ wild-type derivative) is grown on a cell carrying a plasmid with the *stf* and *tfa* genes of Ur-λ, about 1% of the progeny make small plaques, and phages from these plaques have tail fibers (data not shown). The medium plaque size that λ wild type enjoys was one of the phenotypes selected in the construction of λ*PaPa* [and one without which the elegant early experiments defining the control of λ lysogeny (*28*), as well as many subsequent genetic experiments on λ, would likely have been much more difficult]. The correlation between small plaque size and presence of side tail fibers implies that the presence of the *stf* frameshift mutation in λ wild type is the result of selective breeding in the laboratory. Whether there are conditions in the wild that similarly give a selective advantage to a λ that lacks the side tail fibers is unknown.

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12. Virion proteins from an SDS gel were electroblotted to a PVDF membrane (Imobilon-P). Stained bands were excised and sequenced (Porton, model 2090E, gas-liquid phase sequencer).
13. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
14. The Coomassie blue–stained gel of Fig. 3 and the autoradiogram of a similar gel of phage labeled with [^{35}S]methionine were scanned in a densitometer. The peak areas were corrected for protein molecular size (stained gel) or sulfur content (autoradiogram) and converted to copies per virion by normalizing to proteins for which this value is known (gpH: 6 per virion; gpB: 12 per virion). The stained gel gave 8.3 copies per virion for gpStf and 12.5 copies per virion for gpTfa; the autoradiogram gave 11.4 copies per virion for gpStf and 9.7 copies per virion for gpTfa.
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32. Supported by NIH grant GM47795 (R.H.). We thank R. Calendar and E. Kellenberger for supplying the early version of K12(λ) and R. Calendar, S. Casjens, F. Eiserling, S. Godfrey, G. Hatfull, D. Kaiser, and C. Peebles for helpful discussions.

19 May 1992; accepted 2 September 1992

Assignment of a Locus for Familial Melanoma, MLM, to Chromosome 9p13-p22

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Linkage analysis of ten Utah kindreds and one Texas kindred with multiple cases of cutaneous malignant melanoma (CMM) provided evidence that a locus for familial melanoma susceptibility is in the chromosomal region 9p13-p22. The genetic markers analyzed reside in a candidate region on chromosome 9p21, previously implicated by the presence of homozygous deletions in melanoma tumors and by the presence of a germline deletion in an individual with eight independent melanomas. Multipoint linkage analysis was performed between the familial melanoma susceptibility locus (MLM) and two short tandem repeat markers, D9S126 and the interferon-α (IFNA) gene, which reside in the region of somatic loss in melanoma tumors. An analysis incorporating a partially penetrant dominant melanoma susceptibility locus places MLM near IFNA and D9S126 with a maximum location score of 12.71. Therefore, the region frequently deleted in melanoma tumors on 9p21 presumably contains a locus that plays a critical role in predisposition to familial melanoma.

There are approximately 32,000 new cases of cutaneous malignant melanoma (CMM) diagnosed annually in the United States alone and 7,800 melanoma-related deaths. U.S. incidence rates for CMM have been increasing more rapidly than for any other cancer except that of the lung (*1*). Between 1973 and 1985 in Utah, the age-adjusted incidence rate for melanoma increased from 6.4 to 14.2 per 100,000 (*2*). Approximately 10% of melanoma cases arise in a familial setting (*3*); these cases are hypothesized to carry an inherited susceptibility to CMM. However, since melanoma may not be expressed in all individuals who inherit such a susceptibility, a proportion of apparently sporadic melanoma cases may also be due to genetic predisposition.

Several different studies have pinpointed a region on the short arm of chromosome 9 (9p) as one involved in the early stage development of melanoma tumors. In an attempt to determine whether the region contained a familial melanoma susceptibility locus (MLM), we examined genetic markers in the candidate region for genetic linkage with MLM using 11 kindreds with multiple cases of invasive CMM. Although the value of linkage studies in localizing homogeneous, fully penetrant dominant disorders has been established (*4*), the value of such studies in more complex disorders is unclear. The mode of inheritance of familial melanoma has not been established; investigators continue to debate the existence of a major gene, the localization of this putative gene, and the relationship between familial melanoma and an associated trait, the dysplastic nevus syndrome (DNS) (*5*). However, given chromosome 9p21 as a candidate region, we analyzed genetic markers from this region in these kindreds with a partially penetrant dominant genetic model and localized a susceptibility locus for familial melanoma.

In this study, 11 extended kindreds with 82 cases of melanoma diagnosed between the ages of 12 and 93 were analyzed. Each kindred is caucasian and of Northern European ancestry. Ten of the kindreds are from Utah (Fig. 1); one (K3346) is from Texas and has been studied for over 20 years (6). The kindreds were selected for the presence

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