Table 1. THE PER CENT SURVIVAL OF IRRADIATED WILD-TYPE AND *uvs*-7 STRAINS

Yeast strains	Dose in ergs/mm² 200	400	1,200	2,000
$n = a$, *uvs*-7	97·7 ± 6·5	45·8 ± 2·0	0·458 ± 0·042	0·00348 ± 0·00116
$n = a$, *uvs*-7	74·3 ± 4·04	32·4 ± 2·7	0·308 ± 0·026	0·00149 ± 0·00057
$2n = a/\alpha$ *uvs*-7/*uvs*-7	86·6 ± 6·7	52·2 ± 1·7	0·0717 ± 0·0061	0·00351 ± 0·00135
WT, *n* (standard curve)	89·1	75·9	37·2	13·5
WT, 2*n* (standard curve)	95·4	87·0	79·4	53·7

The values are given with 2 × standard errors.

Table 2. THE PER CENT SURVIVAL OF AN IRRADIATED *uvs*-7 HAPLOID STRAIN WITH AND WITHOUT PHOTOREACTIVATION, ± 2 × *S.E.*

Light	Dose in ergs/mm² 600	800	1,200
Ultraviolet alone	49·9 ± 1·15	37·0 ± 0·99	6·44 ± 0·13
Ultraviolet + 30 min white	54·8 ± 1·0	29·3 ± 0·88	13·6 ± 0·57

the results given in Table 1. Both diploid values were in reasonable agreement. It can be seen that the homozygous diploid does not, indeed, show a better survival than either haploid parent. The survivals of wild-type haploid and diploid yeasts, given for comparison, are taken from standard curves. A haploid strain was tested for photoreactivability by giving photoreactivating light for 30 min after irradiation. The results are given in Table 2. Photoreactivation has no effect at the two lower doses. Increased survival at the 1,200 erg dose can be accounted for by the DNA component of killing. Snow's mutant *uvr*-4, which has diploid sensitivity equal to haploid sensitivity, differs from *uvs*-7 in being cross-sensitive to nitrous acid[5].

We conclude that the properties of *uvs*-7 constitute *prima facie* evidence for ultraviolet sensitivity through a mechanism other than faulty DNA repair.

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Isolation and Characterization of a Streptolydigin Resistant RNA Polymerase

THE RNA polymerase of *Escherichia coli* consists of two small subunits α, two larger subunits β and β′, and one subunit σ which aids initiation[1]. A question relevant to control of polymerase synthesis is whether genes coding for the various subunits are contiguous. Both rifampicin resistant RNA polymerase and streptovaricin resistant RNA polymerase map near the argH locus[2,3]. Here I report that (*a*) streptolydigin inhibits chain elongation by *E. coli* RNA polymerase as was found by Siddhikol *et al.*[4] for the polymerase of *B. megaterium*, (*b*) mutants altering the polymerase to streptolydigin resistance also map near argH, and (*c*) that, as is the case of rifampicin resistance[1,2], the "core" enzyme (the $\alpha_2\beta\beta'$ structure which itself possesses enzymatic activity) is modified in the resistant mutant.

E. coli is not normally sensitive to rifampicin or streptolydigin, presumably because it is impermeable to the drugs. To obtain strains with resistant polymerases it was therefore necessary to select sensitive, and presumably permeable, mutants. This was done in two steps, first by selecting a strain permeable to rifampicin, and then selecting a mutant of this which was permeable to streptolydigin. Strain PA607[5] was mutagenized with nitrosoguanidine[6] and, after segregation, rifampicin was added to 100 μg/ml. and penicillin to 2,000 units/ml. In these conditions growing cells are killed, but any cells inhibited by rifampicin are spared. After 1 h the drugs were removed. Three cycles of this treatment yielded a population in which 10 per cent of the cells were inhibited by rifampicin at 20 μg/ml. The same mutagenesis and enrichment procedure was then used to select a mutant sensitive to streptolydigin.

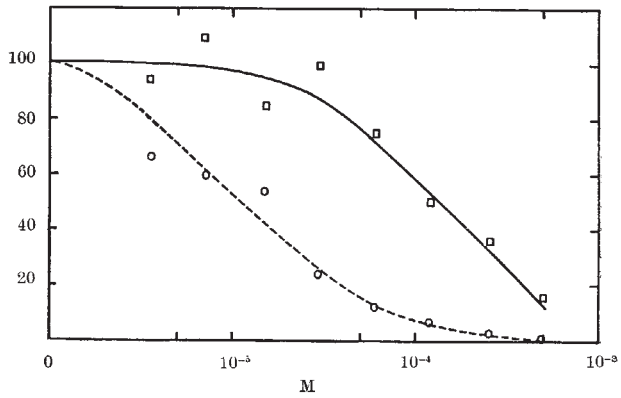

Fig. 1. The activity of "core" RNA polymerase purified from a strain sensitive (○ - - - ○) and resistant (□—□) to streptolydigin in various concentrations of the drug. The polymerase was purified by C. Goff and R. Burgess[1]. It was verified by polyacrylamide gel electrophoresis that less than 2 per cent of the polymerase molecules contained the σ subunit. In these assays 10 μg of polymerase is added to 0·10 ml. reaction mixture containing the indicated concentration of freshly dissolved streptolydigin and *tris*-HCl *p*H 8·0, 0·04 M; $MgCl_2$, 0·01 M; β-mercaptoethanol, 0·002 M; ATP, GTP, CTP, $2{\cdot}5 \times 10^{-4}$ M; [^{14}C]-UTP, 2 μCi/mmole, 10^{-4} M, and calf thymus DNA at 75 μg/ml. For assays on crude extracts K_2HPO_4 at 4×10^{-4} M was included to inhibit polynucleotide phosphorylase. The samples were incubated 10 min at 37° C and 3 ml. 5 per cent TCA containing 0·01 M sodium pyrophosphate was added. The precipitates were collected on 'Millipore' HA, 0·45 μm filters, rinsed with TCA-pyrophosphate, dried and counted. The inhibition does not depend on salt concentration, for the same inhibition is seen with 0·20 M KCl present in the assay mix.

Mutants were then selected to be resistant to streptolydigin by plating a nitrosoguanidine treated culture on plates containing 100 μg/ml. of the drug. Resistant clones were of two classes: those which had become impermeable to the drug and those which had an altered polymerase. The polymerase activity of one of these strains was resistant to streptolydigin, in crude extracts and when 90 per cent pure, thus proving that streptolydigin interacts with polymerase to inhibit its activity. The "core" polymerase purified from strains sensitive or resistant to streptolydigin is just as sensitive or resistant to streptolydigin as the polymerase assayed in crude extracts. Fig. 1 shows the inhibition of sensitive and resistant "core" polymerases.

The location of the mutation conferring streptolydigin resistance to polymerase was found to be very near argH by cotransduction. Phage P1 prepared on the streptolydigin resistant strain was used to transduce a strain to argH plus. In two out of nine such transductants, the RNA polymerase activity assayed *in vitro* in crude extracts was resistant to streptolydigin, thus demonstrating the cotransduction of argH and streptolydigin resistance.

It is interesting to note that the addition of streptolydigin to functioning polymerase halts further chain elongation whereas the addition of rifampicin or streptovaricin halts chain initiation[2,4,7] as shown in Fig. 2. One possible mode for inhibition of chain elongation is a competition between triphosphates and drug for the active site. This does not seem to be the case, because increasing or decreasing the triphosphate concentration by a factor of

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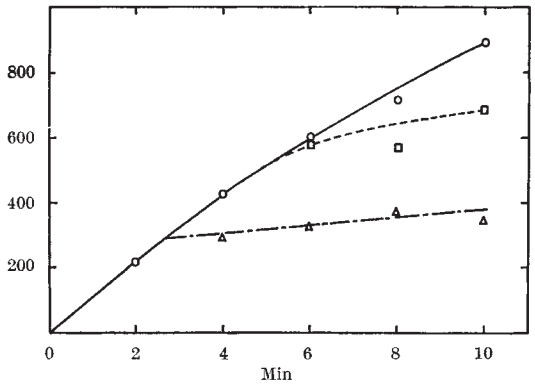

Fig. 2. The time course of RNA synthesis after addition of streptolydigin or rifampicin. Abscissa is the duration of the reaction and the ordinate is counts per min per 0·10 ml. reaction mix. The enzyme was "core" $\alpha_2\beta\beta'$ and the assay conditions were as in Fig. 1. ○—○, Normal reaction; □ - - - □, rifampicin added at 2 min to a concentration of 2 μg/ml.; △— · —△, streptolydigin added at 2·5 min to a concentration of 10^{-4} M. There was no detectable RNA synthesis in a fourth reaction containing rifampicin to which polymerase was added at 1 min.

four did not change the inhibition curve from that shown in Fig. 1. This suggests that the drug and the triphosphates are not competing for the same site. It remains to be shown which subunits are altered in the mutants resistant to rifampicin, streptovaricin and streptolydigin.

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Functional Independence of F and I Sex Pili

CONJUGATION and gene transfer in *E. coli* and other Enterobacteria depend on the formation by donor cells of specialized filaments which may be the channel by which genes pass to the recipient[1]. These "sex pili" fall into two principal classes: "F pili" determined by the F sex factor[1] and by related F-like sex factors[2] found in *fi*+ drug resistance (R) factors and colicin (Col) factors V and B; and "I pili" determined by the I sex factors of ColIa-CA53, ColIb-P9, ColEla and *fi*− R factors[2,3]. The two classes of sex pilus seem to be distinct in many respects[2], and this lack of relationship is further emphasized by the failure of an intact sex factor of one class to complement a mutant factor of the alternative class in pilus synthesis.

Mutant sex factors can be selected by exposing cultures of donor bacteria to donor-specific phage[4]. Such cultures, however, are phage-sensitive initially only if they carry a sex factor which is de-repressed in respect of pilus synthesis so that most cells form sex pili and adsorb the phage[5]. The factor may be de-repressed either in the wild type state, as is F, or following mutation[6], like the de-repressed mutant *fi*+ R factor, R1*drd*-19, which fails to form repressor although remaining sensitive to repression (A. Frydman and E. Meynell, unpublished results). Selection for phage-resistance might therefore yield two types of mutant factor: those defective in pilus synthesis (*psa*−) and those which are *psa*+ but fail to form pili because pilus synthesis has again become repressed. *Cis-trans* tests for repressor synthesis require that the mutant sex factor can readily coexist stably with a second repressible sex factor in the same cell; that is, superinfection immunity[2,7] is absent. This is feasible with F and a *fi*+ R factor but not with any pair of I sex factors so far examined, such as ColIa and ColIb (ref. 7). Phage-selected clones are therefore denoted here by an asterisk until their identity is established (for example, F*).

Thirteen independent F* mutants were isolated from *E. coli* (*Flac*), strain AB1353, by phage MS2. All are believed to be *psa*−, for none repressed R1*drd*-19. That is, wild type F does not seem to have a latent repressor region. Forty-two I* mutants were selected by I specific phage[8]. All were derived from de-repressed mutants of Fredericq's complex plasmid consisting of ColIb-P9 linked to the *trp cys* region of *E. coli*[9]. Twenty-six of the mutants were derived from IP9*drd*-5 *col-trp*+ and sixteen from IP9*drd*-5 *colIb*+ *trp*+. Although superinfection immunity prevented a direct test for repression by these I* mutants, they were examined indirectly, as follows. De-repression of an I sex factor is recognizable not only by an increase in conjugating ability but also by examining broth cultures of their hosts which have been shaken overnight, for these have a smaller optical density and greater colicin titres than those of the same strain carrying the wild type factor. The sixteen I* derivatives of IP9*drd*-5 *colIb*+ *trp*+ were examined in this way: however, they did not fall into two discrete groups corresponding to the de-repressed and wild type plasmids but formed a continuous series between these extremes.

Complementation in pilus synthesis was tested by introducing several unrelated de-repressed I factors (IP9*drd*-5, R144*drd*, R163*drd*, R538*drd* and R64*drd*) into strain AB1353 carrying each of the F* mutants. Similarly, wild type F*lac* or R1*drd*-16 was tested in strains M616 or CL194 carrying the I* mutants. In no case did complementation occur, as shown by the failure of the F* or I* strains to become sensitive to F or I phage, respectively, when drops of phage stock were applied to overlays inoculated with the test strains.

The two types of sex pilus thus seem to be completely distinct by every criterion applied so far. Not only are they structurally dissimilar in their morphology, antigenic structure and phage receptors[2,3,8], but they are also functionally unrelated, as shown by the lack of complementation reported here, the differing specificities of their repressors[2], and by the independent transfer of their factors when both are present in the same host[10].

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