Escherichia coli Mutant Lacking 4-Thiouridine in Its Transfer Ribonucleic Acid

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A mutant of Escherichia coli has been isolated that lacks 4-thiouridine, a rare base in transfer ribonucleic acid. The mutant grows at the same rate as wild-type cells. It shows little near-ultraviolet-induced growth delay, thus supporting earlier hypotheses that 4-thiouridine in transfer ribonucleic acid is the chromophore for this growth delay.

4-Thiouridine (‘Srd) is a modified nucleoside present in the transfer ribonucleic acid (tRNA) of many Escherichia coli strains (1, 5). Its presence does not drastically affect the functions of tRNA in vitro (10, 12). There are no reports, however, on the effect of ‘Srd in vivo. In this note we report the isolation of a mutant of E. coli that has little if any ‘Srd in its tRNA. We find that it grows at the same rate as wild-type cells. The mutant also shows only slight near-ultraviolet (near-UV)-induced growth delay.

‘Srd in tRNA absorbs maximally around 340 nm (4). Irradiation at 340 nm of such tRNA in vitro or in vivo leads to the formation of ‘Srd-Cyd adducts (2, 9), which have been shown to affect the ability of some tRNA’s to be acylated in vitro. Formation of such ‘Srd-Cyd adducts in tRNA, leading to an effective amino acid starvation and cessation of RNA synthesis, appears to be the cause of growth inhibition induced by near-UV radiation (300 to 380 nm) in E. coli (6, 8). Relaxed (rel–) mutants of E. coli, which continue net RNA synthesis during amino acid starvation, also continue RNA synthesis after near-UV irradiation (unlike rel+ strains) and show only minimal growth inhibition (6, 8). This difference in growth response of rel+ and rel– strains to near-UV irradiation can be used to select for relaxed mutants (7).

While standardizing the procedure for the isolation of rel– mutants from E. coli B/r NC32 (lac–, valSsu, rel+) using near UV, we found 1 isolate (out of 16) that was resistant to growth inhibition but, contrary to expectation, was also rel+ by the criterion of RNA accumulation during amino acid starvation. This prompted us to examine the ‘Srd content of this mutant, named E. coli B/r RJ.

tRNA extracted from E. coli B/r RJ showed no detectable absorption around 340 nm. Sodium borohydride reduction of tRNA extracted from RJ cells irradiated with black light (peak emission, 355 nm) failed to reveal the development of a new absorption peak at 385 nm, characteristic of ‘Srd-Cyd adducts (3, 9). Irradiated reduced tRNA from RJ cells did not show (Fig. 1) the 385-nm fluorescence excitation maximum or the 440-nm fluorescence emission characteristic of the reduced ‘Srd-Cyd adduct (3). Based

Fig. 1. Fluorescence excitation (emission at 436 nm) and emission (excitation at 396 nm) spectra (uncorrected) of tRNA extracted from near-UV-irradiated E. coli B/r NC32 (—−−−) and E. coli B/r RJ (------) and then reduced by NaBH4. Cells were grown in M9 minimal medium and irradiated with a General Electric 15-W BLB black-light lamp. tRNA was extracted and reduced with NaBH4 in cacodylate buffer, using procedures of Ramabhadran et al. (9). Both preparations had an optical density of 2.8 at 260 nm, with a 1.0-cm optical path. Fluorescence spectra were recorded on a Hitachi-Perkin-Elmer MPF-2A fluorescence spectrophotometer.

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on the fluorescence spectrum, we estimate that, if ‘Srd-Cyd adducts are present in the irradiated tRNA of RJ, they represent less than 5% of those in wild-type cells. The loss of absorbance of tRNA at 340 nm and the inability to detect ‘Srd-Cyd adducts in near-UV-irradiated tRNA strongly suggest the absence of ‘Srd from the tRNA of the RJ strain.

That the loss of ‘Srd is due to a base substitution in the 8-position (where ‘Srd is found) seems very unlikely, as this would require mutations at identical positions in a number of tRNA genes. ‘Srd seems to be formed in tRNA by post-transcriptional modification (4), and a more plausible explanation is that the mutation affected the modifying enzyme.

The RJ mutant has the same growth rate as the parent NC32 in M9 minimal medium (doubling time, 60 min at 30°C), suggesting that the loss of ‘Srd does not confer any disadvantage under these conditions of growth. Furthermore, strain RJ shows even less near-UV-induced growth inhibition than most of the relaxed mutants we have examined.

Lastly, the existence of a mutant both lacking ‘Srd and being resistant to near-UV-induced growth inhibition provides strong support for earlier proposals (6, 8, 9, 11) that ‘Srd is the chromophore for near-UV-induced growth delay.

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LITERATURE CITED


