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Thymineless death in *Escherichia coli* mutants deficient in the RecF recombination pathway

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Received September 10, 1987

Accepted March 16, 1988

NAKAYAMA, K., SHIOTA, S., and NAKAYAMA, H. 1988. Thymineless death in *Escherichia coli* mutants deficient in the RecF recombination pathway. Can. J. Microbiol. **34**: 905–907.

Like *recF* and *recQ* mutants studied earlier, two other classes of *Escherichia coli* mutants defective in the RecF conjugal recombination pathway, *recJ* and *recO*, were found to be partially resistant to thymineless death. In contrast, a *recN* mutant, also belonging to the pathway, was indistinguishable from the wild type with respect to thymineless death.

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Tout comme les mutants *recF* et *recQ* étudiés précédemment, deux autres classes de mutants d'*Escherichia coli* défectifs au niveau du sentier de recombinaison par conjugaison RecF, *recJ* et *recO*, ont semblé partiellement résistants à la mort par manque de thymidine. Par contre, un mutant *recN*, appartenant lui aussi à ce sentier, était indistinguable du type sauvage en ce qui a trait à cette mort par absence de thymidine.

[Traduit par la revue]

When *Escherichia coli* cells are deprived of thymine in an otherwise complete medium, viability loss of unknown etiology takes place (Cohen and Barner 1954). This phenomenon, known as thymineless death (TLD), is not specific to *E. coli*; virtually all types of cells so far examined, procaryotic or eucaryotic, are subject to TLD. This suggests a common molecular event, perhaps related to some fundamental process such as DNA metabolism, which is responsible for the formation of thymineless lethal damage in many living systems. Recently, a potential clue to its mechanism emerged from our observations (Nakayama *et al.* 1982, 1984) that *E. coli recF* and *recQ* mutants, both deficient in the RecF conjugal recombination pathway (Horii and Clark 1973; Nakayama *et al.* 1984), are partially resistant to TLD. Such resistant mutants would be of value because they might help identify those genes that participate in the generation of the lethal damage. It is therefore interesting to look at TLD sensitivity of other mutants defective in the RecF recombination system. There are five other classes of such mutants: *recA, recJ, recN, recO,* and *ruv* (see Kolodner *et al.* 1985 for a recent review). Of these, *recA* mutants are normal with respect to TLD (Nakayama *et al.* 1982), while *ruv* mutants are hypersensitive to TLD (Iyehara-Ogawa and Otsuji 1984). Here, we describe the TLD phenotypes of the three remaining classes of the RecF pathway mutants. Our results indicated that both the *recJ* and *recO* mutations conferred TLD resistance, whereas the *recN* mutation did not affect TLD.

The *rec* strains used were constructed by P1 transduction of strain KD2197, an isogenic derivative of AB2497 (for the genotype of the latter strain see Bachmann 1972), with JC12123 *recJ284*::Tn*10* (Lovett and Clark 1984), RDK1540 *recN1502*::Tn*5* (Kolodner *et al.* 1985), and RDK1541 *recO1504*::Tn*5* (Kolodner *et al.* 1985) as donors. (The JC and RDK strains were provided by Dr. A. J. Clark and Dr. R. Kolodner, respectively). Without a known phenotype detectable in a *recBC*$^+$ *sbcB*$^+$ background, confirmation of the *recJ*::Tn*10* genotype for each presumptive *recJ* transductant

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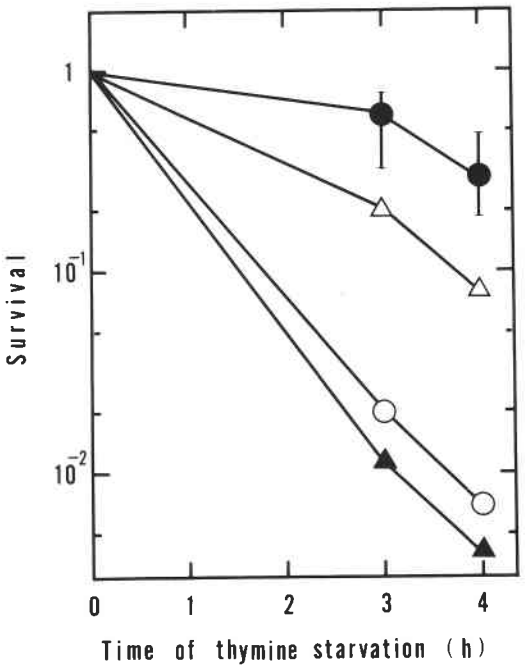

FIG. 1. Thymineless death in *recJ*, *recN*, and *recO* mutants. ○, KD2197 *rec*$^+$; ●, KD2238 *recJ284*::Tn*10*; ▲, KD2239 *recN1502*::Tn*5*; △, KD2240 *recO1504*::Tn*5*. Vertical bars represent the ranges over which survival data for the nine independent *recJ* transductants were distributed. In constructing the test strains, Tc^r and Km^r transductants were selected at drug concentrations of 5 and 30 μg/mL, respectively.

required, for example, a second transduction with a *recBC sbcB* strain as recipient followed by examination of resulting transductants for the Rec$^-$ phenotype. We did not do this, however, because the low transposition frequency for Tn*10* (Kleckner *et al.* 1977) was thought to make the recovery of *recJ*$^+$ tetracycline-resistant (Tc^r) transductants very unlikely. Instead, we examined nine independent Tc^r transductants for TLD sensitivity. The *recN* and *recO* mutations, by contrast, are known to render the cells mitomycin C sensitive (MC^s) and ultraviolet light sensitive (UV^s), respectively, even in a *recBC*$^+$ *sbcB*$^+$ background (Picksley *et al.* 1984; Kolodner *et al.* 1985). Hence, we examined each kanamycin-resistant (KM^r) transductant for such sensitivity to ensure the *recN*::Tn*5* or *recO*::Tn*5* genotype. In the case of *recN*, all of the 16 KM^r transductants obtained were MC^s, whereas 19 out of 20 such transductants were UV^s for *recO*. This latter result is not surprising, since the frequency of transposition for Tn*5* is known to be substantial (Kleckner *et al.* 1977). TLD sensitivity was determined as described (Nakayama *et al.* 1982).

Typical results are shown in Fig. 1. All of the nine presumptive *recJ* transductants, as represented by KD2238, were clearly more resistant to TLD than the *rec*$^+$ parent KD2197. It is thus highly likely that the observed resistance is a phenotype expressed by the *recJ*::Tn*10* allele. KD2240, a representative of the *recO* transductants, also showed increased TLD resistance but to a lesser extent than the *recJ* strains. Eight other randomly selected *recO* transductants gave similar results (data not shown). In contrast, KD2239 representing the *recN* transductants was indistinguishable from the wild type with respect to TLD sensitivity; comparable results were also obtained with seven other randomly chosen *recN* transductants (data not shown).

Since TLD appears to be dependent on the ongoing metabolism of the thymine-deprived cells (Cohen and Barner 1954), strains with very slow growth rates might possibly be somewhat resistant to TLD. We consider it unlikely, however, that this possibility accounts for the observed TLD resistance of the *recJ* and *recO* mutants, because the mass-doubling times as measured by turbidimetry were 65, 72, 76, and 75 min for the *rec*$^+$ (KD2197), *recJ* (KD2238), *recN* (KD2239), and *recO* (KD2240) strains, respectively, under the standard growth conditions used in the present study (Nakayama *et al.* 1982).

These and previous data have established that a subset of the RecF pathway mutants (*recF*, *recJ*, *recO*, and *recQ*) show increased resistance to TLD as compared with the wild type, which indicates a systemic commitment of the RecF-related functions in the lethal event. It is likely that operation of part of the RecF pathway in the absence of thymine may somehow lead to the formation of the hypothetical thymineless lethal damage. DNA double-strand breaks might be involved in TLD (Yoshinaga 1973; Ayusawa *et al.* 1983). Obviously, RecA-dependent homologous pairing and strand exchange between DNA duplexes should not be involved in this hypothetical process, since *recA* mutants are no more resistant to TLD than wild type (Nakayama *et al.* 1982). The indifference of the *recA* mutation also seems to indicate that the RecA-dependent SOS induction, known to involve the *recN* (Lloyd *et al.* 1983), *recQ* (Irino *et al.* 1986), and *ruv* (Lloyd *et al.* 1984) genes, is not relevant to TLD.

It is also important to note that none of the RecF pathway mutations have conferred complete TLD resistance. This could be interpreted to mean that some lethal process other than the one related to the RecF pathway may also be operative in thymine-deprived cells. For example, incorporation of uracil into DNA in place of thymine followed by abortive base excision repair as suggested by Makino and Munakata (1978) would account at least in part for that portion of TLD.

Acknowledgements

We are grateful to Drs. A. J. Clark and R. Kolodner for providing bacterial strains. We also thank Ms. C. Yoshida for support services.

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Coliphages and enteric viruses in the particulate phase of river water

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Received September 23, 1987

Accepted February 26, 1987

PAYMENT, P., MORIN, E., and TRUDEL, M. 1988. Coliphages and enteric viruses in the particulate phase of river water. Can. J. Microbiol. **34**: 907–910.

The present study was undertaken to determine if indigenous enteric viruses and coliphages are free or associated with suspended particulate matter in natural waters. River water was filtered on filters of decreasing porosities (100–0.25 μm) that were pretreated with detergent to eliminate viral adsorption while retaining particulates. This filtered water was refiltered in virus-adsorbing conditions to retain free viruses. The virus-adsorbing filter retained most of the enteric viruses (77.4%) and coliphages (65.8%), which indicated that these viruses were probably free or associated with particles with a diameter of less than 0.25 μm. These observations are important because in water treatment plants small particulates are often the most difficult to eliminate.

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Cette étude avait pour but de déterminer si les virus indigènes entériques et les coliphages sont libres ou associés à des particules en suspension dans un milieu hydrique naturel. L'eau de rivière a été filtrée sur des filtres de porosité décroissante (100–0,25 μm) prétraités avec un détergent afin d'éliminer l'adsorption non spécifique des virus tout en retenant les particules. L'eau ainsi filtrée a été refiltrée en conditions adsorbantes pour recueillir les virus libres. C'est le filtre adsorbant a retenu le plus grand nombre de virus entériques (77,4%) et de coliphages (65,8%). Les virus présents dans l'eau de rivière seraient donc libres ou associés à des particules ayant un diamètre de moins de 0,25 μm. Ces observations sont importantes puisque les particules de cette dimension sont difficile à éliminer lors du traitement des eaux destinées à la consommation.

Human enteric viruses discharged by waste water outfalls or waste water treatment plants are often associated with suspended particulates. This association protects viruses from exposure to environmental factors and water treatments. Because the efficiency of water treatments in eliminating viruses is dependent on their capacity to eliminate suspended particles and solids, it is important to consider these adsorption phenomena.

Previous studies indicate that in treated waste water the majority of enteric viruses are free or attached to particles smaller than 0.3 μm (Hejkal *et al.* 1981). In treated sewage effluent the largest quantity of solid-associated coliphages are attached to particles greater than 8.0 μm and less than 0.65 μm (Gerba *et al.* 1978). Metcalf *et al.* (1984) show that enteroviruses and rotaviruses in estuarine water adsorb preferentially to particles smaller than 0.3 μm in diameter. However, the distribution of enteric viruses in river water has never been studied, even though the water serves as a source

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