POSSIBLE HOMOLOGY BETWEEN GENES FOR TWO AMINOGLYCOSIDE NUCLEOTIDYLTRANSFERASES, AAD(3″) AND ANT(2″): AN EVOLUTIONARY RELATIONSHIP?

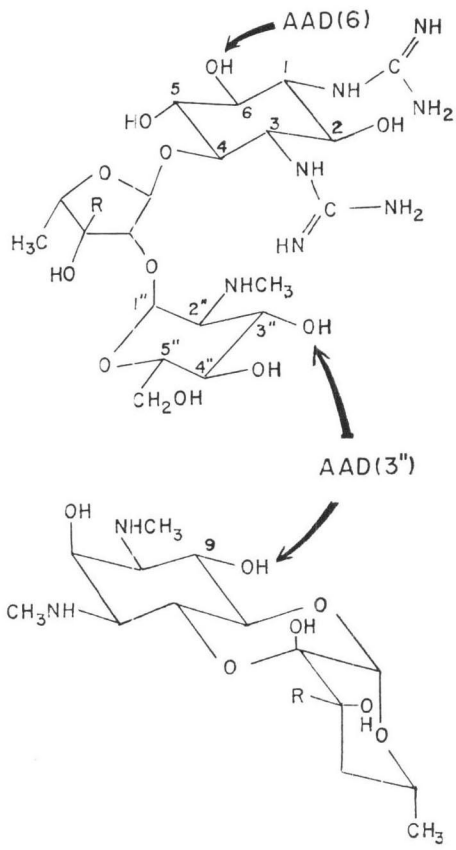
Sir:

Four different plasmid-determined aminoglycoside nucleotidyltransferases have been identified in clinically isolated resistant bacteria. As seen in Fig. 1, aminoglycoside 3″-adenylyltransferase [AAD(3″)][1] and 6-adenylyltransferase [AAD(6)][2] transfer the adenylyl moiety of ATP (or deoxy-ATP) to the 3″- or 6-hydroxyl group of streptomycin. AAD(3″) has also been shown to adenylylate spectinomycin at the 9-hydroxyl group[3]. Aminoglycoside 2″-nucleotidyltransferase [ANT(2″)][4,5] and 4′-nucleotidyltransferase [ANT(4′)][6] have been shown to transfer a nucleotide moiety from a nucleoside (or deoxynucleoside) triphosphate to the 2″- or 4′-hydroxyl group of the 2-deoxystreptamine antibiotics (Fig. 2). AAD(3″) has been found in a variety of Gram-negative and Gram-positive organisms (Table 1); AAD(6) has also been detected in *Staphylococci*. ANT(2″) is one of the most common forms of gentamicin/tobramycin resistance in nosocomial *Klebsiella* infections. ANT(4′) has been found in clinically isolated *Staphylococci* resistant to tobramycin and amikacin, but sensitive to gentamicin and dibekacin.

As an aid to the purification and study of aminoglycoside-modifying enzymes, we are in the process of preparing small multicopy plasmids carrying the genes for these enzymes by recombinant DNA techniques. The gene for ANT(2″) has been cloned[7] onto a mini-colicin E1 plasmid (pVH51) from two different R-plasmids, pJR66-b and pJR207. Fig. 3 shows *Eco*R1 restriction digestion and agarose gel electrophoresis profiles of the recombinant plasmids; pMY26 obtained from the DNA fragments of pJR66-b, and pMY18 obtained from those of pJR207. Both recombinant plasmids contain 4×10^6 dalton fragments corresponding to the D-fragment of pJR 66-b (pMY26) and the F-fragment of pJR207 (pMY18), respectively. In addition, the D-fragment of pJR207 (4.6×10^6) has been demonstrated by gene cloning and DNA-RNA hybridization studies to carry the second copy of the gene for ANT(2″). It is not known if the gene for ANT (2″) is on a transposable element.

In hybridization studies with the complementary RNAs (cRNA) prepared from the recombinant plasmids, pMY26 and pMY18, using the SOUTHERN nitrocellulose blotting method[8], we found evidence for sequence homology with other R-plasmids not coding the gene for ANT (2″), but for AAD(3″). In fact, as shown in Fig. 4 and Table 2, the *Eco*R1 fragments G of R-plasmids R100 and

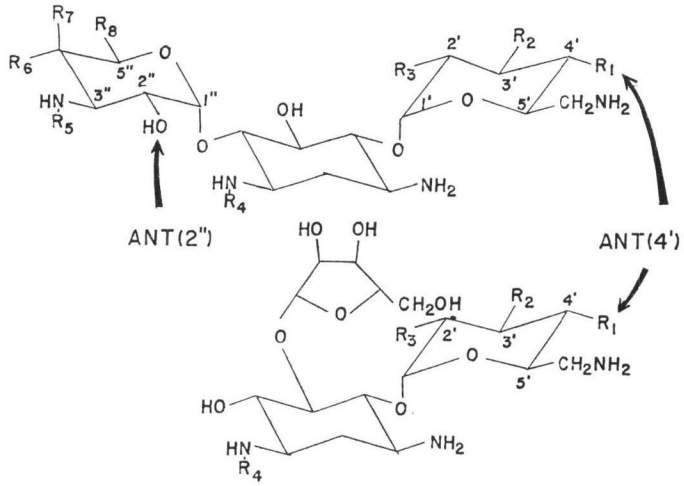
Fig. 1. The substrate aminoglycoside antibiotics for AAD(3″) and AAD(6).



	R
Streptomycin	CHO
Dihydro-streptomycin	CH_2OH

	R
Spectinomycin hydrate	OH
Dihydro-spectinomycin	H

Fig. 2. The substrate aminoglycoside antibiotics for ANT(2″) and ANT(4′).



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Kanamycin	OH	OH	OH	H	H	OH	H	CH_2OH
Amikacin	OH	OH	OH	AHB	H	OH	H	CH_2OH
Tobramycin	OH	H	NH_2	H	H	OH	H	CH_2OH
Dibekacin	H	H	NH_2	H	H	OH	H	CH_2OH
Gentamicin C_{1a}	H	H	NH_2	H	CH_3	CH_3	OH	H
Ribostamycin	OH	OH	NH_2	H	—	—	—	—
Butirosin B	OH	OH	NH_2	AHB	—	—	—	—

AHB: s-4-Amino-2-hydroxybutyryl.

Table 1. R-Plasmid-determined aminoglycoside nucleotidylating enzymes.

Enzyme	Origin	R-plasmid	Substrate antibiotic
Aminoglycoside 3″-adenylyltransferase[AAD(3″)]	*Shigella* *Salmonella* *Escherichia* *Staphylococci* *Streptococci*	R100, R5 R6 ML1629* pAT5 pJH1	Streptomycin Spectinomycin
Aminoglycoside 6-adenylyltransferase[AAD(6)]	*Staphylococci*	MS27*	Streptomycin
Aminoglycoside 2″-nucleotidyltransferase [ANT(2″)]	*Klebsiella* *Pseudomonas*	pJR66, pJR207 POW*	Kanamycin Tobramycin Gentamicin
Aminoglycoside 4′-nucleotidyltransferase [ANT(4′)]	*Staphylococci*	APO1* PAL* STE* FK109*	Amikacin Tobramycin Butirosin

* Strain number is indicated, the plasmid in the strain has not been named.

Fig. 3. Agarose gel electrophoresis pattern of *Eco*R1 fragment of parent R-plasmids, the recombinant plasmids and mini-colicin E1 (pVH51) plasmids.

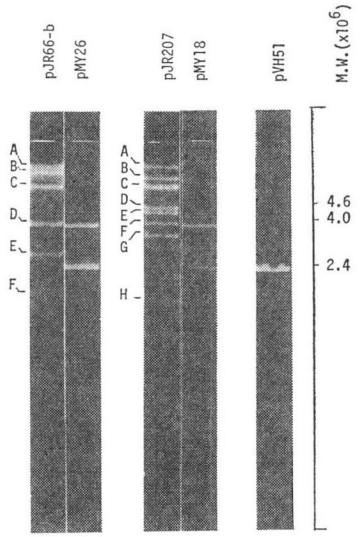


Fig. 4. Hybridization of cRNA of recombinant plasmid with R-plasmid DNA.

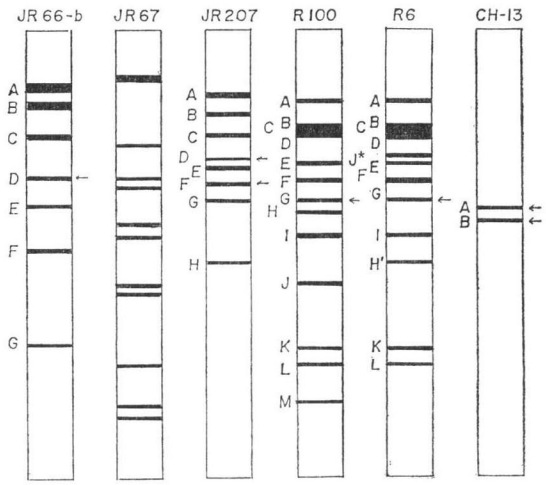


Table 2. Hybridization of the complementary RNAs of pMY26 and pMY18 with EcoR1 fragments of R-plasmids.

R-plasmid	Hybridization of cRNA with fragment	Aminoglycoside inactivation enzyme for resistance to		
		Tm-Gm	Sm	Nm
pJR66-b	D	ANT(2″)	APH(3″)	APH(3′)-II
pJR67	—	—	APH(3″)	APH(3′)-II
pJR207	D, F	ANT(2″)	AAD(3″)	APH(3′)-I
R100	G	—	AAD(3″)	—
R6	G	—	AAD(3″)	APH(3′)-I
CH-13	A, B	—	AAD(3″)	—
pAT5	—	—	AAD(3″)	APH(3′)-III
pJH1	—	—	AAD(3″)	APH(3′)-III

Tm-Gm; tobramycin-gentamicin, Sm; streptomycin, Nm; neomycin, APH; aminoglycoside phosphotransferase.

R6 showed hybridization with the cRNA of pMY26 and pMY18; both G-fragments have been shown to contain the sequence coding for the adenylyltransferase AAD(3″). In addition, cRNA prepared from the recombinant colicin E1-plasmids, CH-13 [constructed with an *Eco*R1 fragment of R-plasmid R5 containing the gene for AAD(3″)], hybridized to the DNA fragment of the parent plasmid, the G-fragments of R100 and R6, the D-fragment of pJR66-b, the fragments D and F of pJR207 and the 4×10^6 dalton fragments of pMY26 and pMY18. The latter five fragments encode ANT(2″).

To summarize our conclusions, pJR66-b and pJR207 both possess *Eco*R1 fragments of identical molecular weight (4×10^6) that encode ANT(2″); in addition, pJR207 possesses a larger *Eco*R1 fragment (4.6×10^6) that encodes the second copy of the same gene. These two R-plasmids, of different compatibility groups, originated in clinical isolates of *Klebsiella pneumoniae* of quite different geographical origins. Radioactive RNAs complementary to these ANT(2″)-containing fragments were found to possess sequence homologies with *Eco*R1 fragments encoding a different aminoglycoside modifying enzyme

AAD(3″), from several sources. In addition, RNA complementary to AAD(3″)-containing fragments hybridized to the DNA of the ANT(2″)-containing fragments.

We have been unable to demonstrate any homology between ANT(2″) or AAD(3″) containing fragments from Gram-negative organisms and R-plasmid DNA from *Staphylococci* or *Streptococci*, encoding the Gram-positive AAD (3″). Thus there appears to be homology between fragments encoding ANT(2″) and AAD(3″) of Gram-negative (*Klebsiella, Shigella* and *Salmonella*) origin, but not Gram-positive.

It is of interest to note that the genes for ANT(2″) on two different *Klebsiella* R-plasmids of separate origin appear to be a common *Eco*R1 fragment of 4.0×10^6 daltons; this implies that the two plasmids share larger regions of homology that contain the fragment encoding ANT(2″). The homologies that we detected between the ANT(2″) and the AAD(3″) fragments may or not be associated with the coding sequence for the enzymes; they may be due to the presence of identical insertion elements (IS) present in DNA fragments encoding these genes in various plasmids. However, there exists the possibility that two R-plasmid-coded enzymes of similar function but different substrate ranges may have related sequences and thus be derived from a common origin. At the present time these enzymes have not been purified to homogeneity and immunological comparisons are not possible.

Acknowledgement

The authors wish to thank the National Institutes of Health (Allergy and Infectious Diseases Institute) for financial support, and M.Y. wishes to thank Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd. for encouragement.

MORIMASA YAGISAWA**
JULIAN E. DAVIES*

* Author to whom correspondence should be directed.

** Present address; Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd. Asahimachi, Machidashi, Tokyo 194, Japan.

Department of Biochemistry
College of Agricultural
and Life Sciences,
University of Wisconsin-
Madison, Madison,
Wisconsin 53706, U.S.A.

(Received December 16, 1978)

References

1) UMEZAWA, H.; S. TAKASAWA, M. OKANISHI & R. UTAHARA: Adenylylstreptomycin, a product of streptomycin inactivated by *E. coli* carrying R factor. J. Antibiotics 21: 81～82, 1968
2) SUZUKI, I.; N. TAKAHASHI, S. SHIRATO, H. KAWABE & S. MITSUHASHI: Adenylylation of streptomycin by *Staphylococcus aureus*: A new streptomycin adenylyltransferase. *In* Microbial Drug Resistance. *Ed.* S. MITSUHASHI & H. HASHIMOTO, Univ. Tokyo Press, 1975
3) YAMADA, T.; D. TIPPER & J. DAVIES: Enzymatic inactivation of streptomycin by R factor-resistant *Escherichia coli*. Nature 219: 288～291, 1968
4) BENVENISTE, R. & J. DAVIES: R-Factor mediated gentamicin resistance: A new enzyme which modifies aminoglycoside antibiotics. FEBS Letters 14: 293～296, 1971
5) YAGISAWA, M.; H. NAGANAWA, S. KONDO, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Adenyldideoxykanamycin B, a product of the inactivation of dideoxykanamycin B by *Escherichia coli* carrying R factor. J. Antibiotics 24: 911～912, 1971
6) SANTANAM, P. & F. H. KAYSER: Tobramycin adenylyltransferase: A new aminoglycoside modifying enzyme from *Staphylococcus epidermidis*. J. Infect. Diseases 134: S33～S39, 1976
7) YAGISAWA, M. & J. DAVIES: Amplified production of an aminoglycoside modifying enzyme by hybrid plasmids. (in preparation).
8) SOUTHERN, E. M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503～517, 1975