

Fig. 2 Schematic representation of synthesis of infectious transducing phage in vitro. In the phage synthesizing system in vitro ΦX174 gene A protein cleaves the viral strand of ΦX174 RFI DNA at its origin of replication (A-site) to form open circular RF (RFII) DNA whose 5'-terminus is covalently attached to gene A protein^{4,5}. The RFII DNA-gene A protein complex then associates with prohead and ss DNA synthesis occurs by a looped rollingcircle mechanism⁴. Host proteins and gene C, gene J proteins of $\Phi X174$ are required in these processes¹ (not shown in figures). The displaced viral strand is encapsidated into prohead while DNA synthesis is occurring. When one round of DNA synthesis is completed, gene A protein cleaves the viral strand and joins the two ends to form a phage particle containing a circular viral strand⁶. Phage particles synthesized in vitro are infectious to ΦX174-sensitive E. coli cells. After infection, the circular ss DNA is converted to ds RF DNA, which is multiplied to produce progeny RF molecules. In the late stage of infection, progeny RF molecules serve as template for synthesis and packaging of ss DNA to produce phage particles by a similar mechanism as in vitro reaction. In the pH24R or pH24L DNA-directed in vitro reaction, Φ X174 gene A protein cleaves either one of the strands of the plasmid DNA at the A-site and forms a plasmid DNA-gene A protein complex. After associating with prohead, asymmetric ss DNA synthesis occurs as in the case of the $\Phi X174$ DNA-directed reaction. Depending on the orientation of the A-site fragment, one of the strands of plasmid DNA is specifically displaced and encapsidated into prohead. Thus pH24R or pH24L DNA-directed reaction produces infectious particles in which complementary circular plasmid ss DNAs are packaged. After infecting \$\PhiX174sensitive E. coli. cells, the plasmid ss DNA is converted to ds plasmid DNA, which is multiplied and maintained in the cell. As a result, tetracycline-resistancy carried by pH24R and pH24L is transformed to E. coli. cells. Neither pH13 DNA nor pACYC184 DNA support synthesis or packaging of DNA due to the lack of the A-site sequence in these plasmid DNAs.

DNA in the transducing particle is converted to and maintained as the ds form in the cell. The maintenance of the transduced plasmid probably occurs via the plasmid replication pathway because it depended on the presence of polA1 protein (data not shown).

The synthesis and packaging of ss DNA from pH24R and pH24L to produce transducing particles also occurred in vivo when cells harbouring these plasmids are infected with wild type $\Phi X174$. The infecting phage apparently provides the

necessary phage-originated proteins to produce the transducing particles by a mechanism similar to that occurring in the in vitro system described in this report. A schematic representation of the in vitro reactions is shown in Fig. 2.

This system will be useful in elucidating the biochemical functions of gene C and gene J proteins. By varying the size of the plasmid carrying the A-site sequence, this system also provides an excellent way to determine the minimal and maximal lengths of ss DNA able to be packaged into the prohead structure-an important parameter for the analysis of phage morphogenesis. The system described in this report also provides some applications in the molecular cloning of DNA. The efficiency of infection of particles containing plasmid DNA (as measured by the transduction of the drug marker) is as high as the efficiency of plating of phage, thereby providing several magnitudes higher transfer efficiency of plasmid DNA into cells over the conventional transformation method. The strand separation of plasmid DNA resulting from its packaging may also be useful in DNA sequencing.

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Errata

In the letter 'A 2-D model calculation of atmospheric lifetimes for N₂O, CFC-11 and CFC-12' by M. K. W. Ko and N. D. Sze, Nature 297, 317-319 (1982), figures 2 and 3 have been transposed.

In the letter 'Helium isotopic systematics of oceanic islands and mantle heterogeneity' by M. D. Kurz et al. Nature 279, 43-47 (1982), on page 45 the first two lines (with age ... degassed of ³He) should be transposed to the bottom of the column.

Corrigenda

In the letter 'Structural alterations in J regions of mouse immunoglobulin λ genes are associated with differential gene expression' by J. Miller, E. Selsing and U. Storb, Nature 295, 428–430 (1982), the sequence of the dJ λ 2 recognition nanomer is incorrect as shown in Fig. 2. The sequence should read GGATCTTGC. This change (underlined) only results in a closer resemblance of the $dJ\lambda 2$ recognition sequence to the consensus, making $dJ\lambda 2$ more like $\psi J\lambda 3$ and, therefore, more likely to interfere with functional $V\lambda 2$ -C $\lambda 2$ joining. Thus, the error does not affect the conclusions presented.

In the letter 'Electrophysiology of mammalian thalamic neurones in vitro' by R. Llinas and H. Jahnsen, Nature 297, 406-408 (1982), in line 4 in the right-hand column on page 407 the term 'early Na conductance' should read 'early K conductance'7.