



Fig. 2. Gel electrophoresis of caulimovirus virion DNA. DNA from purified CaMV virions was electrophoresed in a nondenaturing minigel and stained with ethidium bromide. M, size markers; ND, nondenatured virion DNA showing typical components resolved as an 8 kbp (left) linear, and open circular (oc) forms, including twisted molecules. D, shows the same DNA as in ND, but denatured by incubation in 50 mM NaOH at 65° for 10 min prior to loading on the gel. This shows the typical pattern of three single-stranded DNA components of virion DNA with sizes of 8 kb, 5.4 kb, and 2.6 kb. Note that, since these forms are migrating as single-stranded DNAs in a nondenaturing medium, they do not run with mobility consistent with their sizes relative to the double-stranded DNA size markers (M).

Fig. 3. (opposite page) 2D gel electrophoresis of CaMV intracellular DNA. Total cellular DNA was isolated from infected plants by phenol:chloroform extraction, under which conditions most of the cellular virion DNA is not purified. The sample thus contains viral replicative forms and minichromosome (supercoiled) DNA. This complex population of molecules can be readily resolved on 2D gels. In the first (nondenaturing) dimension, DNAs are separated according to size and conformation. Then, in the second (denaturing) dimension at 90° orientation relative to the first, molecules are resolved largely according to single-stranded size. Theoretical migration of various forms is shown in the upper diagram. The sample well is to the top left. Molecules with equivalent relative mobility in both dimensions migrate along a line we call the unit diagonal (UD). Double-stranded linear molecules (L) fall on this line according to size. Open circular molecules (OC) with linear components resolve as slowly migrating forms in the neutral dimension, but are separated into their various single-stranded forms in the second dimension. For instance, conventional OC molecules are resolved into an 8 kb linear and a more slowly migrating closed single-stranded circular form (filled OC spots); CaMV virion DNA is resolved into the three single-stranded components (half-tone OC spots), as described in the legend to Fig. 2. Supercoiled (SC) forms migrate more rapidly in the first dimension. Smaller SC molecules form a diagonal to the lower right of the main SC DNA. Hairpin (HP) molecules migrate as double-stranded linear forms in the first dimension, but at half their double-stranded mobility when denatured, since they melt to form single-stranded molecules of twice the double-stranded size. Native single-stranded forms migrate along a diagonal between the UD and the HP diagonal. The lower part of the figure shows an actual separation of CaMV DNA forms. For further explanation of 2D gels and their interpretation, see refs. 21 and 22.