Circular Permutation Analysis of Phage T4 DNA by Electron Microscopy

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Phage T4 is known to have a linear duplex chromosome that is circularly permuted and terminally repeated. We found, by denaturation and self-reannealing experiments, that circular permutation in T4 native DNA is not random. Their multimodal distribution of permutation is compatible with the "headful packaging" model with the additional specifications that the encapsulation of DNA starts at several sites and these are not random distributed.

Introduction

Phage T4 chromosome is a linear double stranded molecule which shows terminal repetition [1, 2]. The circularity of the genetic map results from the circular permuteness of the ends of mature, linear DNA [3]: genes that are distant from each other in some DNA molecules are close in other ones.

The DNA inside the phage head reflects the mechanism by which the mature DNA is processed or cut from the overlength concatameric replicative DNA precursor [4].

According to the current model [5] the length of DNA molecule is determined by the amount of DNA which can fit into the head (headful model): if headfuls are cut from the concatameric precursor at different points, circular permutation and terminal repetition as well result; deletions of inessential genetic material are compensated in headful DNA packaging by more extensive terminal repetitions [6]. The model, however, leaves apart the cutting mechanism of the concatamer, and does not take into account the possible interference with the regulation mechanism involved in T4 early gene expression [7].

When after denaturation and self reannealing [8], circularly permuted molecules form homoduplex circles, two pairs of single-stranded branches corresponding to the terminal repetition appear. The distance between the two pairs of single-stranded branches gives the size of the shift of the two reannealed single strand molecules due to the permutation. The distribution of such a permutation length can give, then, information on the cutting mechanism of the concatamer.

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We have measured the s.s. branche distances on a sample of about hundred DNA molecules: the distribution of permutation we found appears multi-modal as to indicate that encapsulation mechanism is not random and several packaging sites can exist.

Materials and Methods

Bacterial and phage strains, media and phage DNA preparation were as described in ref. [9].

a) Denaturation and renaturation of DNA samples

A solution [10] 80% redistilled formamide, 0.4 M NaCl, 0.01 M PIPES buffer, 1 mM EDTA pH 6.5 containing 1 µg/ml of native T4 DNA was incubated for 5 min at 68 °C and then for 90 min at room temperature. Under these conditions, about half of molecules was circularized and 90% of them showed the two pairs of s.s. branches.

b) Electron microscopy and measurements

Sample preparation for electromicroscopy, E.M. pictures and DNA measurement were accomplished as described in ref. [11].

Results

The circumference (L_c) and the length (L_r) of each of the two pairs of s.s. branches (a', b', a'', b'' in Fig. 1) of about hundred circularized molecules were measured. It has to be noted that the lengths of the s.s. branches generally are different each other. The obtained distributions are shown in Figs. 2 and 3 respectively. The width of the L_r distribution is larger than the expected one on the basis of measurement uncertainties, reflecting probably, the

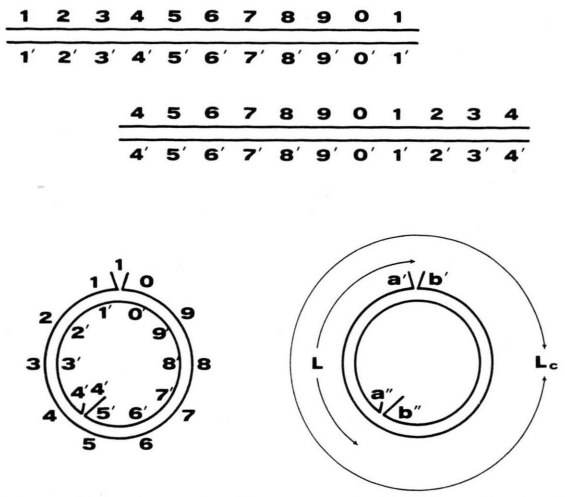

Fig. 1. Experimental scheme. Each DNA molecule is shown having a different circular permutation of a common sequence and a terminal repetition of its first sequences. This can produce circular molecules by chain separation followed by random association and formation of the duplex structure. The circumference on the circle is equal to the phage genome length (L_c); the terminal repetition length (L_r) is given by the average value of the $L'_r = a' + b'$ and $L''_r = a'' + b''$. The whole length of DNA is $L_c + L_r$. The distance (L) between the two pairs of single-branches gives the measure of the staggering in permutation between the two reannealed molecules.

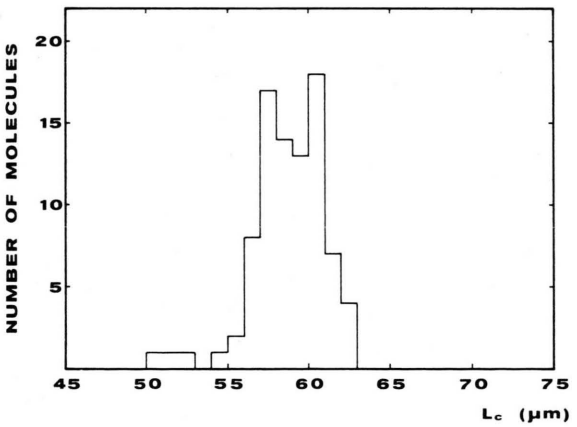

Fig. 2. Contour length for T4 DNA circular molecules. Each bar correspond to 1 μm class size. The mean value of the distribution is $L_c = (58.9 \pm 0.2)$ μm.

variability of the terminal repetition [6]. The mean values of the distributions are $L_c = (58.9 \pm 0.2)$ μm and $L_r = (1.20 \pm 0.04)$ μm in good agreement with the values reported in literature [12]. The distances L (see Fig. 1) around the circle between the positions of the two pairs of s.s. branches were also measured and the resulting histogram is shown in Fig. 4. In the abscissa the values of $R = L/(L_c + L_r)$ are reported. Such values range between 0 and 0.5 as it was chosen the shorter distance.

Now, since the T4 concatamer length ranges from two to three times the mature DNA length [13], and the terminal repetition is approximately the 2% of the DNA length [12], we can expect [14] that the permutation distribution will be:

1. uniform, if the encapsulation mechanism is random;
2. discrete but spanning over a few per cent of the genome length, if the encapsulation mechanism is sequential from an unique starting site;
3. discrete, but spanning over a large part of genome length, if the encapsulation mechanism is not random and several starting sites exist.

Our distribution has a large range and it appears to be discrete with a broad background: the encapsulation mechanism then, is not random.

Moreover we can say that the more evident peaks show that some groups of permutation are more frequent, while some part of genome never permutes. A rough evaluation (Fig. 4) indicates that this part of genome is about the 25% (~ 15 μm). All that suggests that only some starting sites of encapsulation are possible and that they are specific.

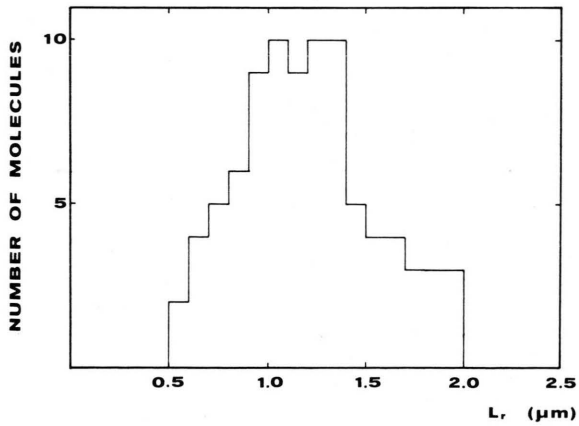

Fig. 3. Length of terminal repetition. For each circularized molecule the average value of the length of the single-stranded branches $L'_r = a' + b'$ and $L''_r = a'' + b''$ on each of the two pairs was measured. Each bar corresponds to 0.1 μm class size. The mean value of the distribution is $L_r = (1.20 \pm 0.04)$ μm.

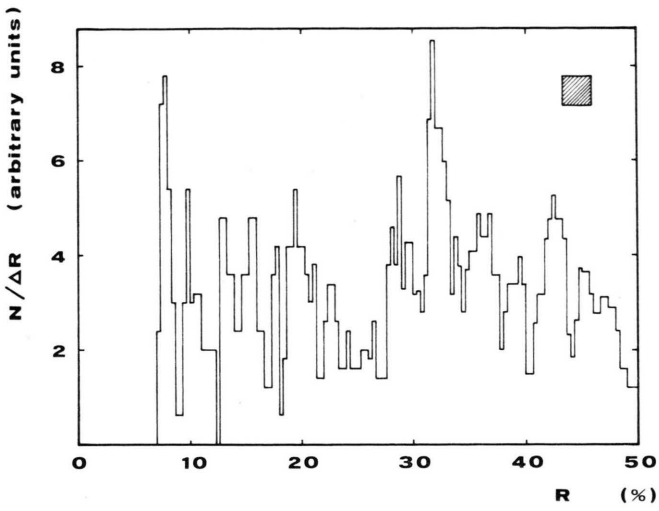

Fig. 4. Single strand branches as a measure of the staggering in permutation between two reannealed single strands T4 DNA. Each measure is represented in the histogram by a rectangle with the base proportional to the experimental error ΔR on the measured length. Correspondingly the leight is proportional to the inverse of the error in order to have the same area for all measures. N is the number of molecules that are in the range ΔR. The square in the insert is the area unit.

Conclusions

Our experimental distribution of permutation, clearly shows a multimodal pattern: this means that the encapsulation mechanism is not random and that only some groups of permutations are possible.

Our result is compatible with the headful model [5], with the additional specification that the encapsulation of DNA starts at several, specific sites.

On the other hand, McHattie *et al.* [8], also found that many different permutations exist in the T2 phage but they had not a sufficient statistics to characterize them.

Our results indicate that some part of the genome does not permute, and this could satisfy the need of not separating the early genes from their promoters [7], a problem until now open in the headful model.

It could be possible, then, to pick out some genes that do not present heterozygotes [2] and, on the other hand, one or more peaks (Fig. 4) would disappear if one or more specific starting sites are deleted as would be possible in some T4 deletion mutants. In this way a genetics characterization of such sites would be possible.

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