Cell biology

Architecture of interphase nuclei

from Virginia A. Zakian

THE orderly appearance and behaviour of chromosomes during mitotic and meiotic cell division must reflect precise and regular three-dimensional associations in the dividing eukaryotic cell. Between divisions (interphase), chromosomes unfold and usually disappear from view, raising the question of whether interphase nuclei possess a specific and predetermined spatial organization. If they do, that organization might influence or even determine the patterns of RNA transcription and DNA replication during interphase. The second stage of an ambitious attempt by John Sedat and co-workers to probe the structure of the interphase nucleus is reported on page 414 of this issue.

Specifically, Sedat's group is investigating the arrangement of polytene chromosomes in interphase cells of the salivary glands of *Drosophila melanogaster* larva. These chromosomes are ideally suited for such studies: successive rounds of endo-replication produce huge chromosomes in which over 1,000 chromatids are exactly aligned and characteristic bands can be correlated with specific genetic loci. Moreover, transcriptional activation occurs at individual loci in a characteristic temporal sequence and can be visualized by the appearance of puffs at specific sites.

The studies of Sedat's group are carried out on intact unfixed cells, using procedures designed to minimize disruption of intracellular associations. Chromosomes are stained with DNA-specific fluorescent dyes and examined by fluorescent light microscopy using sophisticated computer-assisted methods. Twenty-four optical 'slices' are taken through each nucleus. An image-processing program handles focus control and the averaging, storage and alignment of images. A computer program integrates the 24 image stacks to produce a trace of each chromosome's position in the nucleus.

The first results, which were based on a single nucleus, revealed that the complexly-folded polytene chromosomes maintain a polarized orientation[1]. That is, the chromocentre, a structure produced by fusion of all the centromeres, is situated at a fixed position on the nuclear membrane relative to the geography of the salivary gland. This position is opposite to that of the telomeres which also contact the membrane. The chromosomes are positioned close to the nuclear membrane, leaving the centre of the nucleus essentially free of chromosomes. These conclusions were similar to those previously obtained by less precise methods (see, for example, refs 2 and 3).

Technological improvements in image-gathering and analysis have now enabled Sedat and co-workers to identify bands throughout the length of each chromosome arm and therefore to identify individual chromosomes[4]. Thus they have been able to determine whether specific cytological loci occupy similar spatial environments within each of six nuclei taken from the same salivary gland. Sedat's group has also introduced methods for describing spatial positions which are more objective and more easily quantified than those used in previous studies. To describe the intra-chromosomal folding patterns of chromosome arms, an intra-distance map is constructed in which the distance between a given cytological locus and every other locus on the chromosome arm is displayed[4]. In the map, the decrease in distance between two non-contiguous sites on a chromosome brought into proximity by folding is graphically illustrated as a point off the diagonal in the intra-distance plot.

The intra-distance maps obtained by Sedat's group for the same chromosome in different nuclei are not identical; for example, the X chromosome is not limited to a specific three-dimensional configuration. However, statistical analysis of folding patterns reveals that there are preferred configurations. Thus, in four of six nuclei, specific regions of the X chromosome are in apposition to each other. Despite extensive folding, individual chromosomes do not intertwine with other chromosomes but appear to occupy separate topological domains.

Inter-chromosomal distances were also measured. That is, it was determined how frequently specific regions on one chromosome arm are next to specific sites on a second. Preferred contacts between different chromosomes were detected but they are of a limited nature. For example, chromosome arm 2R often lies close to arm 2L but rarely near arm 3L. However, although 2L is found near 2R in all six nuclei examined, the specific sites which are involved in these close contacts differ from nucleus to nucleus.

Finally, by determining the distance from each locus on each chromosome to the nuclear membrane, the specificity of membrane contact points was explored. Most of the chromosomes lie within 5 μm of the nuclear surface (nuclear diameter is 30–40 μm), but the position of many loci vis-à-vis the membrane varies considerably from nucleus to nucleus. Certain chromosomal sites are, however, tightly associated with the membrane in all or most nuclei. The sites correspond to a subset of those loci previously identified as sites of ectopic pairing (pairing between non-homologous chromosomes), late replication and susceptibility to breakage[5]. Note that specific membrane contact points, like intra-chromosomal folding patterns, though conserved, are not invariant features of interphase chromosomes.

Although genes were first viewed as discrete independent loci, it became apparent more than 50 years ago that the position of a gene can have a profound effect on its expression, lending credence to the hypothesis that the interphase nucleus is a highly ordered structure. However, since many chromosomal rearrangements are tolerated in *Drosophila*, nuclear architecture is likely to be flexible except, perhaps, for a limited subset of crucial loci. Sedat's approach has convincingly demonstrated that computer-assisted cytological studies can provide a wealth of positional information at the level of the individual genetic locus. The emerging picture is one of preferred, but not required, chromosomal configurations. To test the importance of these configurations, it will be crucial to determine whether the three-dimensional structure of polytene nuclei changes as a function of developmental stage or genetic makeup. □

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On 30 March 1984, the Marine Biological Association of the United Kingdom celebrates the centenary of its formation.

100 years ago

THE SOCIETY FOR THE BIOLOGICAL INVESTIGATION OF BRITISH COASTS

THE meeting held for the purpose of inaugurating a new society having the above title, took place last Monday in the rooms of the Royal Society, Prof. Huxley being in the chair.

Prof. Huxley began by observing that the establishment of marine biological stations had been undertaken during the last few years by most of the civilised countries, and was, indeed, a necessary result of the great change which had taken place in the aims of biological science. The study of development began about half a century ago, and the ramifications of that inquiry, which had been extended to the mode of becoming of all live things by Mr. Darwin, had caused a complete change in the methods of biological research. In order to investigate the living being it was now no longer deemed sufficient, as in the days of our great-grandfathers, to observe its outside, or even in the days of our grandfathers, to examine its anatomy. We have now to trace its developmental growth from the egg, and we are able to do so with a thoroughness of which no one in his young days could have had any conception. Such was one good reason for founding an institution of this kind from a purely scientific point of view.

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