Extending the capabilities of interphase chromatin mapping

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The last several years have witnessed a revolution in the ability to detect single copy genes and DNA segments with high resolution by non-isotopic *in situ* hybridization[1-5]. Not only does this have important applications for mapping specific sequences on metaphase chromosomes, but several studies have now shown that fluorescence detection of sequences in less condensed interphase chromatin can provide exceptionally high resolution analysis of closely spaced DNA sequences[1,2,4,5]. While the distance between a given pair of probes is variable from nucleus to nucleus, the average interphase distance has a linear correlation with DNA distances up to at least 1 megabase (Mb)[2,4,5]. Hence, as initially described[1,5] two different strategies can be used to estimate sequence order: comparison of the average distance between different pairs of hybridization signals or scoring the configuration of three or more signals visualized simultaneously within the nucleus. This is proving to be valuable for determining the order and relative proximity of sequences from the same chromosomal segment, greatly expanding the range of applications to which *in situ* hybridization can be applied.

The utility of interphase mapping has best been demonstrated in the 0.1–1 Mb range, but what is the potential of this mapping technique above and below this range? Two recent reports[6,7] describe different approaches to address this and to extend the utility of interphase mapping. While previous studies indicated a roughly linear correlation of interphase distance to DNA distance in the range up to 1 Mb[2,4,5] work by van den Engh *et al.*[6] contributes a more thorough analysis in which they show that interphase DNA from 0.1 to almost 2 Mb follows a random walk model. In essence, this indicates that in this range the chromatin fibre behaves similarly to a flexible polymer without constraints that can adopt a large number of random configurations, strengthening the idea that on average the interphase distance between two sequences can be used to predict DNA distance. Results agree with previous estimates of an accuracy for each pairwise hybridization of about 150 kilobases (kb) in the several hundred kb range[4], but further show that accuracy decreases significantly between 1-2 Mb, with the linear correlation (and "random walk") essentially lost above this. Although the error of individual pairwise measurements is relatively large, results from van den Engh *et al.*[6] indicate that the confidence of distance estimates can be increased substantially by measuring the average distance between any given probe and several (rather than one) other probes, thereby building a consensus map based on numerous pairwise combinations. This is a valuable strategy which the authors argue could be applied to large numbers of probes to create a 100 kb physical map of a chromosome.

If less condensed interphase chromatin provides greater resolution than metaphase chromosomes, then why not push the limits of resolution further by decondensing or stretching out the nuclear DNA? There are several methods whereby one can extend nuclear DNA, and the particular approach used is probably critical to the outcome. Heng *et al.*[7] have hybridized single-copy probes to what they term "free chromatin" released from nuclei with drugs (AMSA or pingyamycin) or alkaline treatment to produce highly elongated, but undefined spindle shaped structures. The structural basis for these large masses of elongated nuclear chromatin is unknown. Heng *et al.* show resolution of sequences spaced 21 kb apart (actually ~45 kb from midpoint to midpoint), estimate that sequences 10 kb apart should be resolvable, and suggest that this approach may significantly enhance the utility of interphase mapping and make the process of ordering more straightforward. However this approach may find limited utility for routine gene mapping, since sequences 50–100 kb apart are already resolvable by "standard" interphase approaches[2,4] and ordering of sequences less than 50–100 kb is often not required by *in situ* mapping. Our current perspective is that the most pressing and frequently encountered challenges for *in situ* mapping lie in the efficient ordering of sequences

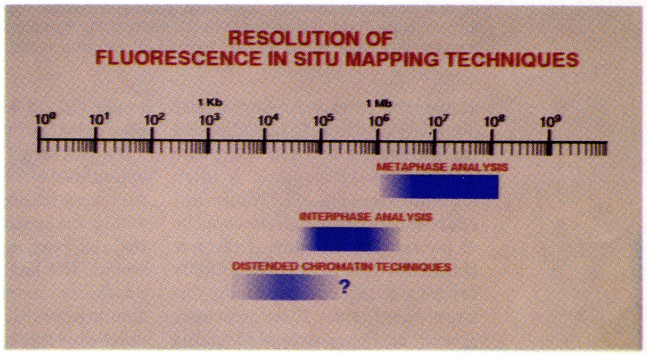

Fig. 1 FISH resolution. Bars represent the range over which each approach is applicable while the lighter areas of each bar indicate the portion in which there is greater variability in applying that particular approach. For example, metaphase analysis is straightforward for resolution of sequences more than 3–4 Mb apart, but requires two colour, two probe analysis on elongated chromosomes to achieve 1 Mb resolution.

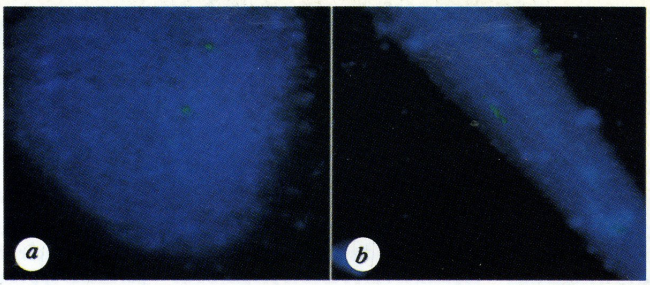

Fig. 2 FISH using a single copy cosmid probe (about 35 kb) in an intact nucleus (*a*) and distended DNA (*b*). Total DNA (blue) and cosmid hybridization (green) are shown. Note in the distended DNA (*b*), each of the homologous copies of the sequence is detected as a linear "string" of punctate signal.

across the interphase–metaphase boundary (the 1–3 Mb range), where the connection between interphase and metaphase mapping is often tenuous (see Fig. 1). While not addressed by Heng *et al.*[7], it would be of interest to determine if the free chromatin structures could simplify ordering of sequences spanning this larger range, since these structures appear as hyper-extended whole chromosomes after chromosome painting and, therefore, may make ordering along the length of the "chromosome" more straightforward.

We have developed an approach[8] coupling *in situ* hybridization with chromatin decondensation methods primarily to investigate the organization of DNA sequences within the nucleus, as well as to explore extending interphase resolution. Different protocols can be used depending on whether the objective is to preserve aspects of chromatin packaging or to extend DNA as much as possible. In nuclei from which histones have been extracted by treatment with 2M NaCl[9] or LIS[10], we find that fluorescence hybridization with a phage or cosmid probe (10–35 kb target sequence) produces a "string" of tiny spots up to a few μm long (Fig. 2). The dotted appearance of the "string" may result from competition of repetitive DNA within the target sequence or from the self-reannealing of the DNA strand. The fact that a 10 kb probe produces a string of signals demonstrates that the resolution is well below this length, as opposed to the free chromatin structures where a cosmid probe of 35–40 kb generally produces just a single spot of signal[7], as it does within the unfractionated nucleus. The high resolution we obtain is sufficient to visualize overlapping cosmids, which could be useful in a subset of gene mapping problems, such as ordering within cosmids contigs. These chromatin decondensation methods, however, may prove most valuable for studies of chromatin organization, for we have observed that different sequences behave dramatically different by this procedure. In cells treated to create nuclear "halos" we find[8] that sequences fall into two classes depending on whether they remain a tightly condensed spot similar to unfractionated nuclei, or distend into a "string" of spots. This procedure can be used to study the differential packaging of replicating DNA, transcriptionally active DNA, metaphase versus interphase DNA and DNA at different stages of the cell cycle[8]. The observation that differences in packaging between DNA are preserved throughout our hybridization procedures lends credence to the view that at least some aspects of native chromatin structure are preserved during hybridization.

It is clear that several different interphase chromatin mapping strategies can be used in a variety of situations to order sequences from the same chromosomal segment. A key question remains as to whether this technology can be applied routinely and efficiently in a broad spectrum of laboratories, or whether it will remain the specialized, expertise of just a few laboratories. Methods for decondensation of DNA may play a more minor role for gene mapping, however methods which unravel chromatin in ways that still preserve some of the native structural associations may be a powerful approach to understanding the packaging of the chromatin fibre, the potential role of repetitive elements, and the relationship of both of these to genome function. □

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