mission is more efficient when signal-producing and signal-responsive cells have achieved a geometry that favors side-to-side and end-to-end interactions between cells. This suggests that C-factor may function as a developmental timer to trigger sporulation only when multicellular aggregates have achieved the highest possible cell density.

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26. *Myxococcus xanthus* nonmotile strain DK4170 contains a *lacZ* fusion gene at position Ω4401 that is expressed in a C-factor–dependent manner (*10, 21*). DK4176 contains a *lacZ* fusion gene at position Ω4455 that is expressed independently of C-factor (*10, 21*). Cells were grown and concentrated to a density of 5×10^9 or 5×10^{10} cells per milliliter (*10*). Higher cell densities failed to produce uniform suspensions. Clone fruiting agar (*16*) was either unsupplemented or supplemented with X-gal (20 μg/ml), dried for 1 hour at 50°C, and scored in one-dimensional strokes with sterilized Al_2O_3 sandpaper (Sears, #925316). Other developmental surfaces allowed less optimal cell alignment. Cells (10 μl) were spotted on scored and unscored areas and incubated at 32°C. After 3 and 5 days, cells were photographed with an inverted light microscope (Leitz). To photograph aligned cells in grooves, cells were spotted on blocks of 2-mm-thick clone fruiting agar that had been dried and scored on glass microscope slides. These agar blocks were maintained at 32°C in a humid chamber (to prevent agar desiccation) for 24 hours, overlaid with a glass cover slip, and photographed under oil immersion with a differential interference contrast microscope at 100× magnification (Zeiss).

27. We thank H. Kimsey for suggesting the use of clone fruiting agar, B. Sager for suggesting the use of agar blocks for light microscopy, and K. Irvine for helpful discussion. S.K.K. is a student in the Medical Scientist Training Program. Supported by NIH grant AGO 2908.

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Interphase and Metaphase Resolution of Different Distances Within the Human Dystrophin Gene

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Fluorescence in situ hybridization makes possible direct visualization of single sequences not only on chromosomes, but within decondensed interphase nuclei, providing a potentially powerful approach for high-resolution (1 Mb and below) gene mapping and the analysis of nuclear organization. Interphase mapping was able to extend the ability to resolve and order sequences up to two orders of magnitude beyond localization on banded or unbanded chromosomes. Sequences within the human dystrophin gene separated by <100 kb to 1 Mb were visually resolved at interphase by means of standard microscopy. In contrast, distances in the 1-Mb range could not be ordered on the metaphase chromosome length. Analysis of sequences 100 kb to 1 Mb apart indicates a strong correlation between interphase distance and linear DNA distance, which could facilitate a variety of gene-mapping efforts. Results estimate chromatin condensation up to 1 Mb and indicate a comparable condensation for different cell types prepared by different techniques.

NONISOTOPIC IN SITU HYBRIDIZATION procedures have been under development because of their greater speed and precision over widely used autoradiographic techniques (*1, 2*). We have previously demonstrated fluorescence in situ hybridization methodology capable of detecting a few kilobases of single sequences in individual metaphase or interphase nuclei (*3, 4*). This high hybridization efficiency and low background resulted from quantitative analysis of in situ hybridization parameters (*3, 5*) applied with the biotin-labeling technique (*2, 6*). In addition to applications for chromosome mapping, previous work (*3*) demonstrated simultaneous resolution in interphase nuclei of two sequences within a single integrated viral genome separated by only 130 kb. This raised the possibility that metaphase and interphase analysis combined could allow physical gene mapping across a broad range of distances, encompassing those approachable by both genetic recombination (*7*) and pulsed-field gel electrophoresis (PFGE) (*8*). The work reported here is based on one-step detection of relatively small genomic sequences (phage clones, 9- to 10-kb inserts) with standard fluorescence microscopy (*9*), without benefit of specialized image processing and computer enhancement techniques.

A sequence may be rapidly localized in terms of its relative position along the length of unbanded chromosomes, as has been recently illustrated for chromosome 11 cosmids (*10*). Cytogenetic banding techniques (including G, Q, or R banding) have been adapted for fluorescence mapping of several human genes (*11, 12*). Figure 1, A to C, provides a direct comparison of the localization of the human *Blast*-1 gene by different approaches. Although both fluorescence techniques provide improved precision and speed over autoradiography, fluorescence detection coupled with banding provided the most accurate and precise placement, independent of chromosome condensation. If measurements were restricted to longer (prometaphase) chromosomes, the range decreased substantially but not, in this case, to the point that it was quite as precise as banding. Moreover, measurements on longer chromosomes placed the gene below the position derived by banding (Fig. 1, B and C). With banding techniques the gene was readily localized to a region encompassing approximately 5 to 7 Mb of DNA. Because banding tends to be time-consuming, we have worked toward rapid simultaneous visualization by two-color fluorescence of bands with hybridization signals (Fig. 1D). Bromodeoxyuridine (BrdU) incorporation

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into G bands was detected by addition of a fluoresceinated antibody to the probe detection step and provides a rapid, convenient chromosome identification (*13*).

To address specifically whether metaphase mapping (*14*) can be used in the 1-Mb range, as has been suggested (*10*), we used the large, well-characterized human dystrophin gene (*15*). Two probes for sequences within the human dystrophin gene separated by 1.1 Mb were simultaneously hybridized to cytogenetic preparations of normal human lymphocytes. In 46% of metaphase figures, especially in more condensed metaphases, these sequences were resolved as two signals separated across the width of each chromatid (Fig. 2A). In the rest of the metaphases, particularly on longer chromosomes, the two signals coalesced into one. Analysis of 15 to 20 metaphases for each of several hybridizations (representing distances of 125, 375, 500, 675, and 750 kb) indicated that sequences separated by ≥400 kb could barely be resolved on each chromatid of more contracted chromosomes. Both the frequency and configuration of "doublet" signals on the chromatid was extremely variable and condensation-dependent. Hence, for the dystrophin gene, sequences separated by 1 Mb were not resolved along the chromosome length and, therefore, could not be ordered. Other observations indicate that the practical limits of metaphase mapping may be closer to a few megabases (*13, 16*). The ability to order closely linked sequences on chromosomes is limited because the chromosome width can encompass substantial lengths of DNA.

Complete mapping of the human genome will depend largely on analysis of sequences not far enough apart to be ordered by chromosome hybridization. Our previous work (*3*) and recent work by Trask *et al.* showing interphase mapping of closely spaced sequences in Chinese hamster nuclei (*17*) indicate the feasibility of this approach. We needed to assess whether average interphase distance would correlate with linear DNA distance, especially in the important 100-kb to 1-Mb range, where the folding and looping of the chromatin fiber (*18, 19*) might distort this relation. Hybridization to two sequences within the human dystrophin gene separated by 1 Mb was investigated within nuclei of normal human peripheral blood lymphocytes (PBLs). As expected for X chromosome sequences, single-probe hybridization to male lymphocytes produced a single hybridization signal in 90% of nuclei. A small fraction of nuclei contained two signals, generally very closely spaced, which represent G_2 cells in which the sequences have been replicated (*20*). In contrast to single-site hybridization, targeting two sites separated by 1 Mb produced two precise, closely spaced signals in the majority of nuclei (Fig. 2C). Paired probes for smaller intervals representing 125, 375, 500, 675, and 750 kb all produced readily resolvable paired interphase signals (Fig. 2D). In contrast, hybridization with two overlapping cosmid clones (Erb-B2) produced only one hybridization signal for each of the two chromosome 17 homologs (Fig. 2F).

To assess whether measured interphase distance correlates with known DNA distance of <1 Mb, we performed blind analyses on coded samples. Initially it was determined that smaller (300 and 375 kb) compared to larger (675 and 750 kb) distances could be discerned by just a brief examination (5 min per slide). For statistical comparison, nuclei were photographed and distances between paired signals measured from negatives enlarged by projection (Fig. 3, A and B). There is a normally distributed intercell variation; however, the larger aver-

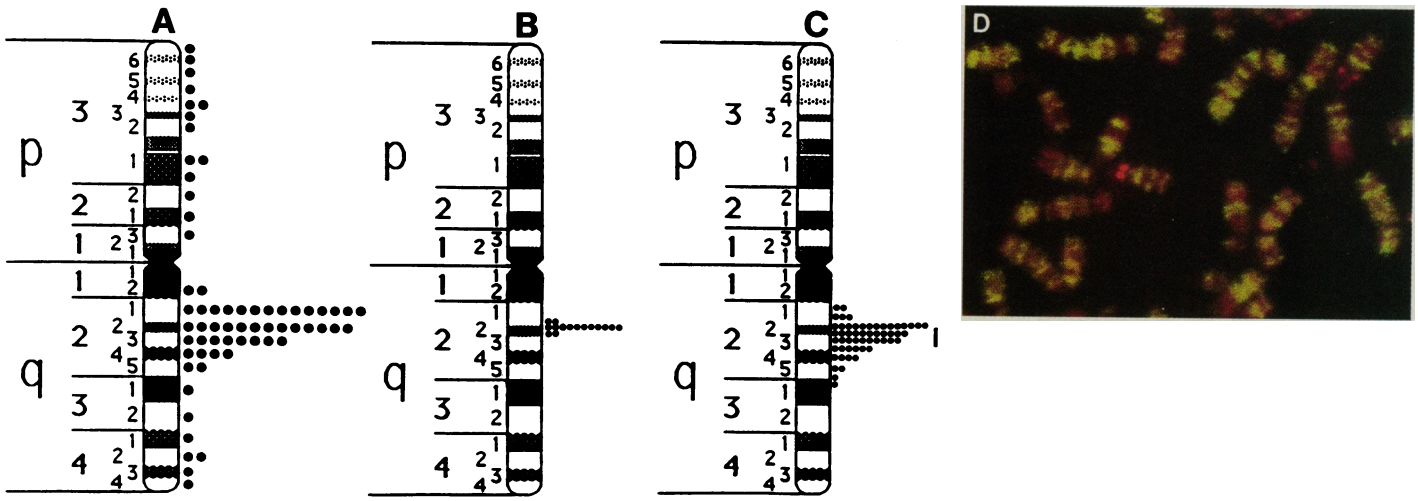

Fig. 1. Comparison of *Blast*-1 gene localization methods. (**A**) Autoradiographic detection on G-banded chromosomes with a ^{3}H-labeled cDNA probe localized this gene to 1q21–1q23, as reported (*11*). [Reprinted from (*11*) with permission, ©1989 Rockefeller University Press.] (**B**) Fluorescence detection on G-banded chromosomes (15 determinations). Metaphase figures were Giemsa-trypsin–banded, photographed, and rephotographed after hybridization with *Blast*-1 genomic phage probes. Total chromosome morphology was visible with fluorescein, allowing precise alignment on a grid of the Giemsa-stained and fluorescein-stained chromosomes; centromeres and telomeres were used as reference points. Although trypsinization weakens hybridization signal slightly, this 20-kb target was still clearly detectable. Dots smaller than those in (A) were used to accommodate more precise placement of fluorescent signals. Despite varying chromosome condensation, this gene consistently localized to the interface of 1q21 and 1q22. (**C**) Fluorescent detection on unbanded chromosomes (55 determinations). Signals were localized by measurement. To correct for chromosome condensation, each data point was expressed as the ratio of the distance from telomere to signal to the total chromosome length. Measurements were made from enlarged photographs by two investigators with essentially identical results. The range of only elongated prometaphase chromosomes (six determinations) (vertical bar) places this gene in distal 1q22–proximal 1q23, just below the band identified by G banding. Some discrepancy in placement between banding versus measurement has been observed for sequences on other chromosomes (*13*). The correlation of measurements to bands may be subject to some inaccuracy because the placement of bands on ideograms is not based on measurement, since relative band position is variable for technical reasons (*25*). (**D**) Simultaneous visualization of signals with G bands identified by BrdU incorporation. This is a single photograph, without computer enhancement or alignment, taken with a single dual-wave length filter (Omega Corp.). One labeled chromosome 1 is in the middle and the other is in the upper right. In lymphocytes labeled for 6 hours before harvesting, late-replicating DNA produces a G-band pattern (*26*) after detection with a fluorescein-conjugated mouse monoclonal antibody to BrdU [Boehringer or Becton Dickinson, (*13*)]. Hybridization was detected with Texas red–avidin. Although not as detailed as high-resolution bands, BrdU bands are rapid high-contrast and simultaneous with hybridization. This circumvents alignment problems inherent in double exposure or computerized superimposition.

age interphase distance for sequences separated by 675 or 750 kb (0.86 and 0.85 μm, respectively) compared to 375 kb (0.66 μm) is evident from the histograms and statistically significant ($P < 0.01$). Consistent with their similar sizes, within the range of error of the PFGE analyses, 675 and 750 kb were not significantly different. Analysis of average interphase distance (Fig. 3B) demonstrated a strong correlation with DNA distance. For the range examined, the overall correlation approximated linearity; however, this must represent a linear region of a curve with a decreasing slope. This is indicated by analysis of sequences separated by 6 centimorgans (cM) (roughly 6 Mb), which were on average 2.81 μm apart, and sequences 70 Mb apart, which were only separated by about 6 μm at interphase (*21*). Although more work is required in the range of several megabases, results for the 1-Mb range indicate that the correlation is sufficient to allow correct ordering of distances differing by, conservatively, 150 kb. Accuracy would be enhanced by analysis of larger cell numbers or, possibly, by use of a highly homogeneous cell population. Importantly, the 1-Mb distance measured in two different cell types (PBLs and G_1-arrested WI-38 fibroblasts, Fig. 3B) yielded very similar interphase distances.

The lower limit of resolution at interphase is clearly less than 100 kb, and several observations indicate that sequences separated by ~25 kb are just at the limit of resolution of fluorescence microscopy (0.1 to 0.2 μm apart). Hybridization with a probe for the human α-cardiac myosin heavy chain gene (*22*) frequently produced very closely paired doublets (see Fig. 2F), presumably because of hybridization of sequences within two highly homologous genes, separated by ~25 kb, and integrated viral sequences separated by 50 kb were often resolvable (*23*). Resolution of ≤250 kb has been recently well demonstrated for the *dfhr* locus of Chinese hamster chromatin (*17*), and distances were found to correlate with DNA distance in this range. Although small distances are more approachable by gel electrophoresis, it is potentially important for ordering efforts that sequences only 25 to 100 kb apart are visually distinguishable from overlapping (Fig. 2F).

Table 1. Changes in packaging ratio (PR) with increasing DNA distance. The PR is calculated as the interphase distance (see Fig. 3) divided by linear DNA distance. The linear distance is determined from the known number of base pairs (derived from PFGE) multiplied by 3.4 Å per nucleotide.

DNA distance (kb)	PR Packaging ratio
125	1:73
375	1:194
500	1:242
675	1:296
1050	1:334
1050 (WI-38)	1:347

Several results contribute new information concerning chromatin and nuclear organization. It was important to determine whether the unexpected resolution between closely spaced sequences was a consequence of the cytogenetic spreading methodology and methanol–acetic acid fixation. Comparable resolution was observed after hybridization to intact monolayer fibroblasts, the nuclei of which had been preserved within the cytoplasm by paraformaldehyde fixation, without prior swelling or flattening of any kind (Fig. 2, E and F). The 1-Mb distance in two different types of normal diploid human cells, PBLs and primary fibroblasts, exhibited remarkably similar condensation despite gross differences in nuclear size and morphology (Table 1), suggesting a fundamental level of chromatin packaging common to nuclei with apparently different higher organization. Further evidence that our measurements reflect chromatin organization is that the condensation for a normal human gene at the 125-kb distance was approximately 1:73, close to the 1:40 to 1:50 packaging ratio (PR) predicted for the 30-nm chromatin fiber (*19*). This is similar to previous measurements of condensation for integrated viral DNA in human cells (*3*) and hamster DNA (*17*). The hybridization process may affect chromatin condensation; however, results are not inconsistent with estimates of overall condensation at interphase of 10^3 or more (*19*) because the distance between close sequences is less affected by higher level folding. The PR increased with DNA

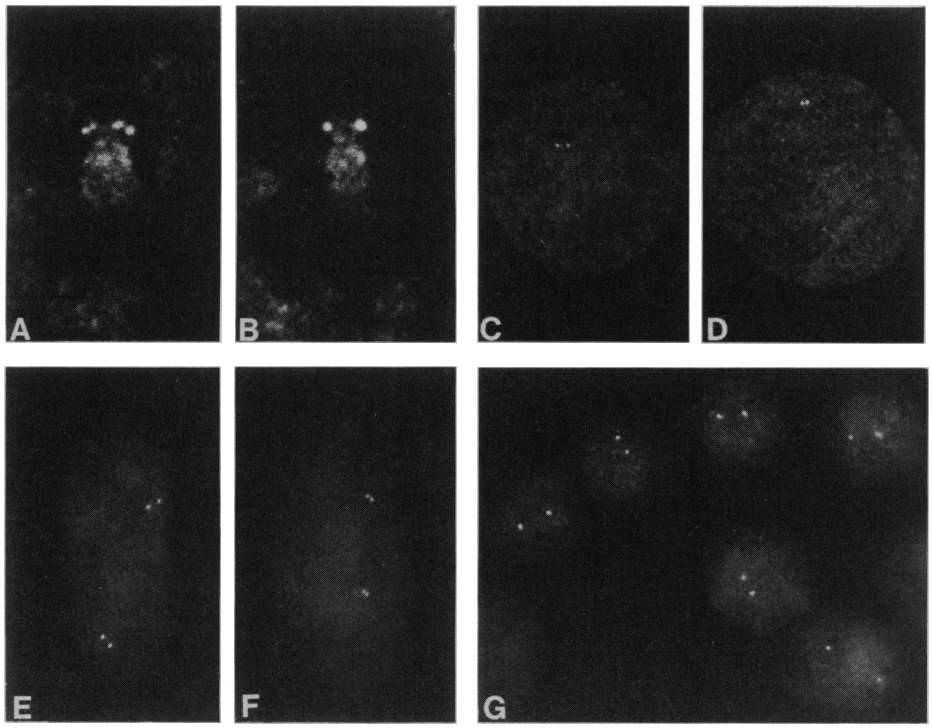

Fig. 2. Simultaneous hybridization of two probes 1 Mb or less apart to cytogenetic preparations of normal human lymphocytes (A through D and G) or paraformaldehyde-fixed human fibroblasts, detected by fluorescein-avidin. Probes were tested individually to assure high hybridization efficiency (80 to 90%) and negligible background. Original magnifications are stated. (**A** and **B**) Resolution of sequences across the width of metaphase chromosomes, ×3300. From two dystrophin probes separated by more than 400 kb, two discrete signals on either side of the chromatid axis are frequently observed (A). Hybridizations with probes separated by smaller distances generally result in one signal on each chromatid (B), as does single-probe hybridization. The distance between signals on each chromatid is variable and related to the degree of chromosome condensation. (A) Probes 1050 kb apart, 845A2 and W14B. (B) Probes 125 kb apart, 16B2 and 24A2. (**C**) Interphase nucleus of male PBLs showing hybridization to two dystrophin sequences separated by 1050 kb (probes 845A2 and W14B). Magnification ×1800. (**D**) Same as in (B) except sequences were separated by 375 kb (probes 16B2 and 30A1). (**E** and **F**) High resolution of sequences within interphase nuclei of intact, paraformaldehyde-fixed WI-38 fibroblasts (derived from a female), magnification ×2500. (**E**) Dystrophin sequences separated by 675 kb (probes W14B and 16B2) are clearly resolvable in ~90% of nuclei. (**F**) Very closely paired signals are frequently (~20%) observed with the α-cardiac myosin heavy chain probe, which is homologous to both the α and β myosin heavy chain genes on chromosome 14. (**G**) Lower magnification view of hybridization to two overlapping cosmid probes for the *neu* protoncogene (*erb*-B2), magnification ×1400. The probes produce one large signal for each chromosome 17 homolog.

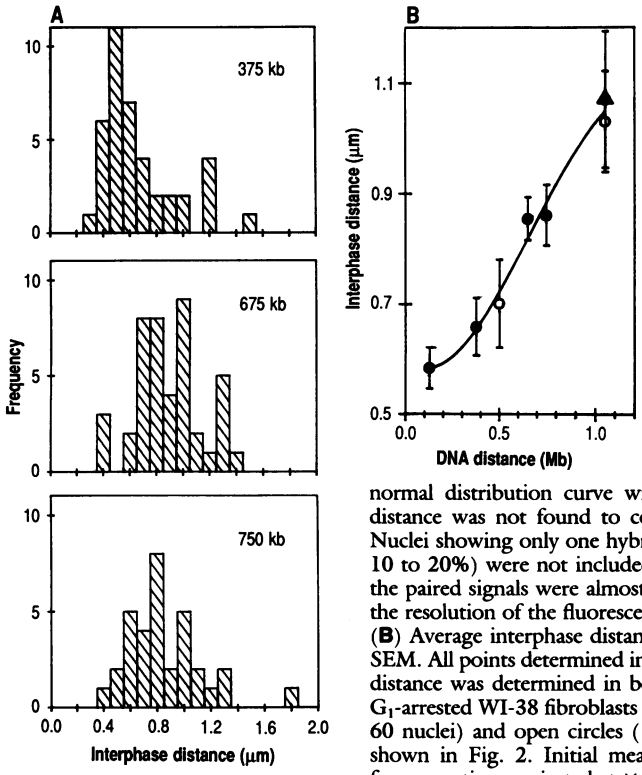

Fig. 3. Interphase analysis of dystrophin sequences phage genomic clones (~10-kb inserts) (*15, 27*) representing the intervals indicated were obtained from L. Kunkel and hybridized using genomic DNA (*9*). The DNA distances are based primarily on estimates from PFGE which agree with work from other laboratories within 5 to 15% (*28*). (**A**) Histograms of interphase distances within individual nuclei of PBLs. A minimum of 35 nuclei was scored from randomly selected, coded photographs. The histograms suggest a normal distribution curve with a defined peak. Interphase distance was not found to correlate with nuclear diameter. Nuclei showing only one hybridization signal (approximately 10 to 20%) were not included (*29*). At the distance studied, the paired signals were almost all >0.4 μm apart, well above the resolution of the fluorescent microscope (0.1 to 0.2 μm). (**B**) Average interphase distance for dystrophin sequences, ± SEM. All points determined in normal PBLs, except the 1-Mb distance was determined in both PBLs (circles) and primary G_1-arrested WI-38 fibroblasts (triangle). Solid symbols (35 to 60 nuclei) and open circles (16 to 20 nuclei). Examples are shown in Fig. 2. Initial measurements were in millimeters from negatives projected at ×6400 and distances presented in micrometers represent actual distance. The curve derived by third-order regression from PBL data is presented for emphasis. Although a linear relation is generally seen in this size range, the larger picture would predict a curve with gradually decreasing slope, as suggested by other observations for smaller (25 to 30 kb ≃ 0.1 to 0.2 μm) and larger (6 cM, roughly 6 kb = 2.81 μm; 70 Mb ≃ 6 μm) distances.

distance as the interphase chromatin fiber folds, up to 1:334 at 1 Mb (Table 1). Our analysis of sequences separated by ~70 Mb on chromosome 1 indicated that the PR increased to 1:2000–1:3000. Since condensation at 1 Mb is still small, the repeating unit of chromatin organization that establishes the greater PR must involve significantly longer stretches of DNA.

In metaphase chromosomes, sequences from 500 kb to 1 Mb apart frequently separate across the chromatid width, suggesting that the chromatin fiber may cross the chromatid axis, and may do so in relatively short stretches of DNA. This work describes the visualization of replicated "sister chromatin" genes closely aligned within S/G_2 interphase nuclei (*20*), consideration of which is essential for interphase mapping and which also demonstrates an approach for precise timing of specific gene replication within single cells. Finally, this work shows that for three genes the homologous sequences are not somatically paired within lymphocytes or fibroblasts (Fig. 2, E, F, and G).

For human genetics, these results, together with other work (*3, 10, 17*), demonstrate that fluorescence in situ hybridization can extend beyond a powerful approach for chromosome mapping, comparable in resolution to somatic cell genetics with deleted chromosomes, to a qualitatively different technology which compares with the fine structure resolution of PFGE (*8*). For many applications, detailed quantitative analysis would not be required, since brief microscopic inspection is sufficient to distinguish between sequences resolvable along the chromosome length versus more tightly linked sequences resolvable only at interphase, or between interphase distances differing by a few hundred kilobases. Moreover, sequence order can be directly ascertained from the configuration of three or more signals within interphase nuclei, as previously demonstrated using intensity "double-label" (*3*). Currently, two-color detection of digoxygenin and biotin labels is being used for sequence ordering after simultaneous hybridization of three probes (*13, 24*).

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9. The hybridization and detection conditions were as previously described (*3*). Probes were labeled with biotin (16)-deoxyuridine triphosphate (Boehringer) (*2, 6*). Digoxigenin(11)-deoxyuridine triphosphate detected by fluoresceinated antibody works equally well (Boehringer). A series of quantitative studies previously identified several key parameters that allowed us to demonstrate, using biotin-avidin, that fluorescence detection could provide single-copy sensitivity, high efficiency, and negligible background (*3, 5*). Among these parameters was the finding that fragment size after nick-translation critically impacted background, hence the deoxyribonuclease (DNase) concentration of the nick-translation reaction was carefully controlled and probe size monitored on agarose gels (*3, 4, 5*). As reported for biotin-labeled probes hybridized to whle cells or tissues, the size range of probe molecules should not extend above 300 to 400 nucleotides (*5*); for cytogenetic preparations, the full range should not extend above ~800 nucleotides (*3, 4*). It was also shown that probe concentrations 100 to 1000 times greater than routinely used for autoradiography were essential for single-copy detection (*3*), that nonspecific sticking of avidin in 4× SSC (standard saline citrate) was reduced to one-tenth that of phosphate-buffered saline (PBS) (*5*), and the preservation of cellular material through storage, hardening, and gentle pretreatments (*3–5*) was essential for optimal results. Cytogenetic preparations were made from phytohemagglutinin-stimulated male PBLs by standard techniques. In some experiments, incorporation of BrdU into DNA before harvesting was used to increase chromosome elongation. Hybridization to repetitive sequences was competed by inclusion of DNase-digested total human DNA (0.5 mg/ml) in the hybridization reaction, similar to the use of C_0T-1 DNA first described for in situ detection of cosmids with interference reflection microscopy [J. E. Landegent *et al.*, *Hum. Genet.* 77, 366 (1987)]. Samples were denatured at 70°C in 70% formamide and 2× SSC for 2 min then hybridized overnight at 37°C in 50% formamide and 2× SSC containing each probe at 2.5 to 5 μg/ml, salmon sperm DNA at 2 mg/ml, *Escherichia coli* tRNA at 2 mg/ml, 10% dextran sulfate, and 1% bovine serum albumin (BSA). After rinsing, samples were stained in fluorescein-avidin at 2 μg/ml in 4× SSC and 1% BSA for 45 min at 37°C. Chromosomes and nuclei were stained with either 4′,6-diamidino-2-phenylindole (DAPI) or propidium iodide and visualized at ×1000 on a Zeiss ICM or Axioplan microscope.
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18. Evidence indicates that the chromatin fiber is packaged in loops, believed to contain on the order of 50 to 100 kb of DNA. See, for example, J. R. Paulson and W. K. Laemmli [*Cell* **12**, 817 (1977)] or (*19*).
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20. In a cycling cell population that contains G_2 cells or in cultures of established cell lines, which generally contain tetraploid cells, a fraction of interphase nuclei will show two signals where one is expected. Replicated signals are generally closely spaced and are indistinguishable from the paired signals produced by simultaneous hybridization of two probes for two different sequences within the gene (see Fig. 3). This was confirmed by analysis of hybridizations with single probes in conjunction with BrdU labeling of S-phase and G_2 cells. This allowed us to determine that ≤11% of the normal human PBLs studied generated paired signals with a single probe and that these were S/G_2 cells. The X chromosome sequences studied here are apparently late replicating; however, this frequency can be much higher when using probes for other regions of the genome. When hybridizing two probes, a small fraction of S/G_2 cells will contain clusters of three to four signals because of the presence of replicated DNA. These cells are easily recognized and not included in the data. The consideration of replicated DNA becomes critical primarily in an analysis of very closely spaced sequences (less than 100 kb), in which resolution of two probes is not observed in most cells, but only 20 to 30%. Hence, replicated and tetraploid cells could constitute a major fraction of data points or actually mimic resolution of closely spaced sequences. We have found that this potential pitfall can be addressed either by BrdU labeling of cycling cells or by use of G_1-arrested cells (see WI-38 1 Mb data, Fig. 3B).
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29. Because single-probe hybridization efficiencies ranged from 80 to 90%, a fraction of nuclei when hybridized with two probes showed only one signal (10 to 30%). These were not included as "zero distance" data points, as they would introduce significant error. The histograms indicate that for distances of 675 and 750 kb, there is no spread of data points into the region where resolution becomes limiting (0.2 μm) and very little for the 375-kb distance. For smaller distances, the exclusion of single-signal data points may introduce some error into the determination of the PR (Table 1), but should still accurately discriminate larger and smaller distances.
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Conserved Sequence and Structural Elements in the HIV-1 Principal Neutralizing Determinant

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The principal neutralizing determinant (PND) of human immunodeficiency virus HIV-1 is part of a disulfide bridged loop in the third variable region of the external envelope protein, gp120. Analysis of the amino acid sequences of this domain from 245 different HIV-1 isolates revealed that the PND is less variable than thought originally. Conservation to better than 80 percent of the amino acids in 9 out of 14 positions in the central portion of the PND and the occurrence of particular oligopeptide sequences in a majority of the isolates suggest that there are constraints on PND variability. One constraining influence may be the structural motif (β strand—type II β turn—β strand—α helix) predicted for the consensus PND sequence by a neural network approach. Isolates with a PND similar to the commonly investigated human T cell lymphoma virus III_B ($HTLV\text{-}III_B$) and LAV-1 (BRU) strains were rare, and only 14 percent of sera from 86 randomly selected HIV-1 seropositive donors contained antibodies that recognized the PND of these virus isolates. In contrast, over 65 percent of these sera reacted with peptides containing more common PND sequences. These results suggest that HIV vaccine immunogens chosen because of their similarity to the consensus PND sequence and structure are likely to induce antibodies that neutralize a majority of HIV-1 isolates.

THE PROGRESS OF HIV-1 VACCINE development has been impeded by the amino acid sequence variability among different isolates of HIV-1. This variability is particularly high in the external envelope protein, gp120, which is the primary target for antibodies that neutralize virus infectivity (*1–3*). The PND of gp120 lies within a loop formed by a disulfide bridge between two invariant cysteines at positions 303 and 338 (*4–7*). Polyclonal antisera elicited by peptides of the PND, as well as monoclonal antibodies that bind the PND, neutralize virus infectivity (*4, 5, 7–9*), and PND peptides absorb most of the neutralizing antibodies elicited by gp120 or its precursor, gp160 (*4*). The PND is one of the more variable regions of the envelope and differs by as much as 50% among HIV-1 isolates (*10*). Because of this variability, neutralizing antibodies elicited by the PND from one isolate generally do not neutralize isolates with PNDs of different amino acid sequence (*4*). To develop a vaccine that elicits antibodies that neutralize a majority of HIV-1 isolates by binding to the PND, it is necessary to analyze PND sequences from a large number of HIV-1–infected individuals.

To obtain the PND sequences, we collected peripheral blood mononuclear cells (PBMCs) from 133 HIV-1–infected donors and cocultured these cells for 14 days with uninfected PBMCs (113 donors) or with H9 or CEM cell lines (20 donors) (*11*). The HIV-1–infected PBMCs were obtained from 95 randomly selected U.S. Air Force personnel from diverse geographic locations or from 38 infected persons in seven major U.S. cities and are likely to be representative of HIV-1 isolates from the United States. Donors were chosen irrespective of clinical symptoms.

Donor PBMCs were cultured with uninfected PBMCs to amplify infectious rather than defective virus genomes. (Similar sequence results were obtained when PND sequences were determined from unamplified virus cultures.) Total cellular DNA was extracted, and the region encoding the PND was amplified by the polymerase chain reaction (PCR) with oligonucleotide primers that hybridize with conserved flanking sequences (*11, 12*). The PCR product was

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