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13. The numerical factor of 3/2 applies to virtually impermeable cells that exclude external fields; as fields penetrate, the factor decreases to 1 (*11*).
14. Self-quenching of fluorescein chromophores by resonance energy transfer or collisions cannot account for the observed results. In fact, extensive cross-linking of FITC-IgER complexes to each other by IgE antiserum does not cause measurable quenching of fluorescein fluorescence (A. K. Menon, D. Holowka, B. Baird, in preparation).
15. The measured fluorescence intensities represent convolution integrations of the source with the instrument point spread function along the optical axis of the microscope. Convolutions of an approximate Gaussian instrumental function with trial fluorescence distributions on the surface of a spherical cell (Eq. 8) showed that the out-of-focus contributions to the measured intensities result in negligible underestimates of f_0 and β by a few percent and an overestimate of I_{im} by a few percent. Our cells are slightly flattened, but the adequacy of the spherical approximation was verified.
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Human Ribosomal RNA Genes: Orientation of the Tandem Array and Conservation of the 5′ End

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The multiple copies of the human ribosomal RNA genes (rDNA) are arranged as tandem repeat clusters that map to the middle of the short arms of chromosomes 13, 14, 15, 21, and 22. Concerted evolution of the gene family is thought to be mediated by interchromosomal recombination between rDNA repeat units, but such events would also result in conservation of the sequences distal to the rDNA on these five pairs of chromosomes. To test this possibility, a DNA fragment spanning the junction between rDNA and distal flanking sequence has been cloned and characterized. Restriction maps, sequence data, and gene mapping studies demonstrate that (i) the rRNA genes are transcribed in a telomere-to-centromere direction, (ii) the 5′ end of the cluster and the adjacent non-rDNA sequences are conserved on the five pairs of chromosomes, and (iii) the 5′ end of the cluster is positioned about 3.7 kb upstream from the transcription initiation site of the first repeat unit. The data support a model of concerted evolution by interchromosomal recombination.

HUMAN DIPLOID CELLS CONTAIN 300 to 400 copies of the genes encoding 18*S* and 28*S* ribosomal RNA (rRNA) (*1*), occurring in blocks of tandemly repeated genes on the short arms of the five pairs (numbers 13, 14, 15, 21, and 22) of acrocentric chromosomes (*2*). Each rRNA gene block (rDNA) lies within the uniquely staining nucleolus organizer region (NOR) (*3*), flanked on the proximal (centromere) and distal (telomere) side by a region of non-rDNA. The 44-kb rDNA repeat unit (Fig. 1) contains a 13-kb transcribed portion and a 31-kb nontranscribed spacer (*4*). The 13-kb RNA transcript is processed to yield the 18*S*, 5.8*S*, and 28*S* mature rRNA molecules. Within a block of rDNA, the repeat units are tandemly arranged in a head-to-tail fashion but the orientation of this cluster on the acrocentric chromosomes has not previously been determined. Little information has been available on the borders of the cluster. In particular, the end unit and adjacent sequence have not been identified, and it is not known whether these end points are conserved among the five acrocentric chromosomes or among individuals in the population. The identification of an X-autosome translocation involving ribosomal RNA genes has allowed us to determine the orientation of the rDNA complex on the acrocentric chromosomes and to clone DNA sequences from the junction of rDNA with sequences on the telomeric side.

The unique translocation t(X;21) (p21;p12) (Fig. 1, inset) between the X chromosome and chromosome 21 was ascertained in a girl with X-linked muscular dystrophy (*5*). The exchange point in the X chromosome is at the muscular dystrophy locus in band Xp21 and the rearrangement is responsible for the disease (*6*). The exchange point in chromosome 21 was mapped cytologically within the rDNA block at band 21p12 (*5*) and molecular analysis revealed that three to five copies of rDNA plus the distal end of chromosome 21 were translocated to the X-derived translocation chromosome, der(X), while 40 to 60 copies of rDNA remained on the chromosome 21–derived translocation chromosome, der(21) (*7*).

These studies were greatly facilitated by the availability of somatic cell hybrid lines containing the der(X) chromosome (hybrid A2) and the der(21) chromosome (hybrid B2), free of other human acrocentric chromosomes (*7*). The estimate of the number of copies of rDNA on der(X) came from quan-

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titative Southern blot analysis of the A2 hybrid line with the rDNA probe B_{ES} (*7*). The B_{ES} probe revealed not only three to five copies of the expected 6.3-kb Bam HI fragment (Fig. 1), but also revealed a weaker band corresponding to a 15-kb Bam HI fragment that was not a feature of the repeating rDNA series. This fragment was considered to be a candidate for a junction fragment spanning either the junction with the X chromosome (XJ in Fig. 1) or the junction of the rDNA block with distal (telomeric) DNA on chromosome 21 (DJ in Fig. 1). The X-junction possibility was ruled out by a number of criteria and the true X junction has since been cloned and sequenced (*6*). The hypothesis that the 15-kb fragment might contain the distal junction from chromosome 21 was supported by quantitative Southern blot analysis that revealed a signal intensity corresponding to one copy per cell in the A2 hybrid line, but about ten copies per cell in normal human DNA, the latter possibly corresponding to the ten acrocentric chromosomes per cell (*7*). The present study was designed to rigorously test this hypothesis by isolation and detailed characterization of the 15-kb fragment.

On the assumption that the 15-kb Bam HI fragment contained the rDNA distal junction, the orientation of the putative junction was determined by probing double digests of human DNA and the A2 hybrid DNA with the B_{ES} probe. The 5′ side of the B_{ES} probe is determined by an Eco RI site (Fig. 1) and a Kpn I site lies immediately on the 3′ side of this probe. The double digests therefore included either Eco RI or Kpn I together with a second restriction endonuclease. Double digests with Eco RI produced bands that conformed to the published map of the rDNA repeat unit. Two bands were visualized in the double digests containing Kpn I and Hind III, Xba I, Bam HI, or Bgl II; there was an intense band corresponding to several hundred copies per cell of a size expected from the rDNA map and a much lighter band derived from fragments present about ten times per human diploid cell. The minor band was not due to a Kpn I polymorphism because the fragment size did not differ from the major band by a constant size differential. Since no further novel fragments were visualized after hybridization of A2 hybrid DNA with many rDNA probes and since the novel fragments visualized with the B_{ES} probe were present at about ten copies per diploid human cell, the simplest interpretation was that the minor bands contained junction fragments from the distal end of the rDNA cluster on each of the ten acrocentric chromosomes. The size of the junction fragments allowed the construction of a restriction map for the putative distal sequences (Fig. 1) and provided an orientation of the complex with the distal region on the 5′ side of the rRNA gene cluster.

To confirm and extend these observations, the 15-kb Bam HI fragment (Fig. 1) was cloned from hybrid A2 and from normal human cells. Two clones (λDJ3 and λDJ15) were obtained from a normal human library (*8*), and two clones (λDJ21-1 and λDJ21-2) were isolated from a similar library constructed from A2 hybrid cell DNA. The 15-kb insert sequences from the clones λDJ3 and λDJ15 were restriction mapped and found to be nearly identical. The map of λDJ15 is shown in Fig. 1 and contains all the sites expected from the Southern analysis plus others not revealed by Southern blot analysis. The order of the six Pst I fragments was determined from double digests and from the fact that the 2.7-kb fragment hybridized with the B_{ES} rDNA probe while the adjacent 2.9-kb fragment hybridized with the C_{BE} rDNA probe (Fig. 1). The remaining four Pst I fragments did not hybridize with any rDNA probe, consistent with a switch from rDNA to the distal flanking sequences in the 2.9-kb fragment. The λDJ3 clone differed from λDJ15 in that it had a 2.8-kb fragment instead of a 2.9-kb fragment, consistent with known sequence variability in this region of the C fragment.

The λDJ21-1 and λDJ21-2 clones, derived from the der(X) translocation chromosome, have not been characterized in detail, but yield six Pst I fragments of the same size as λDJ15. Because λDJ3 and λDJ15 were obtained from a library of normal human DNA they could be derived from any of the five pairs of acrocentric chromosomes, whereas λDJ21-1 and λDJ21-2 must be derived from the distal region of chromosome 21.

To further characterize the junction region, each of the six Pst I fragments from λDJ15 and λDJ3 was subcloned into a plasmid vector. The detailed restriction map of the 2.9-kb fragment was compared to the equivalent region of the C_{BE} clone of rDNA and was found to match over a 2.5-kb stretch (Fig. 1), thus narrowing the putative junction to a 0.4-kb region at the 5′ side of the 2.9-kb fragment. The 5′ end of this fragment was sequenced and compared to the C_{BE} clone of rDNA. The sequence change indicative of the junction occurs

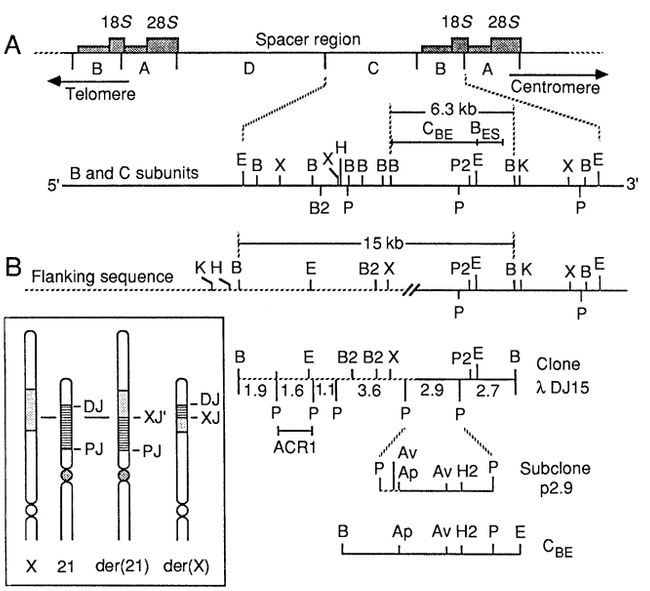

Fig. 1. (**A**) Schematic of the ribosomal gene repeat unit. The stippled block represents the 13-kb transcribed portion; the 18*S* and 28*S* sequences are shown as raised blocks. The smaller 5.8*S* sequence is not shown. The repeat unit contains four Eco RI sites that release the four fragments labeled A (7.3 kb), B (6.2 kb), C (11.7 kb), and D (19 kb). Below the schematic is the restriction map of the B and C region (*13, 4*). The probe C_{BE} is a Bam HI–Eco RI fragment of the C region, and B_{ES} is an Eco RI–Sal I fragment of the B region. The 6.3-kb Bam HI fragment revealed by B_{ES} is shown. (**B**) Restriction map of the putative distal junction region as determined from genomic Southern blots. Ribosomal sequences are represented by a solid line and the adjacent sequences by a dashed line. To generate the map, human genomic DNA was digested with Kpn I plus one of the following: Pst I, Pvu II, Xba I, Bgl II, Bam HI, or Hind III. The cleaved DNA was fractionated by electrophoresis on agarose gels, blotted to nitrocellulose, and probed with the rDNA probe B_{ES}. The Eco RI site was mapped by probing an Eco RI digest with probe C_{BE}. Below this Southern blot map is the restriction map of λDJ15 isolated from a human genomic library (*8*). The map was obtained by a series of single and double digests of the Bam HI released 15-kb insert. A more detailed map was constructed by subcloning each of the six Pst I fragments in a plasmid vector. The map determined for the 2.9-kb Pst I fragment is shown for detailed comparison with the map of the C_{BE} segment shown below. Enzymes are abbreviated as: Ap, Apa I; Av, Ava I; B, Bam HI; B2, Bgl II; E, Eco RI; H, Hind III; H2, Hind II; K, Kpn I; P, Pst I; P2, Pvu II; X, Xba I. The inset shows a schematic of the t(X;21). The Duchenne muscular dystrophy locus is in band Xp21 (stippled area) and the block of tandemly repeated rDNA is in band 21p12 (horizontal stripes). The translocation junctions are marked XJ and XJ′ and the natural junctions of rDNA with non-rDNA are labeled DJ (distal junction) and PJ (proximal junction).

within the 2.9-kb Pst I fragment 155 bp from the 5′ Pst I site (Fig. 2). This Pst I site is approximately 3350 bp from the Eco RI site separating the B and C subunit (Fig. 1). The transcription initiation site is 513 bp downstream from this Eco RI site. Thus the ribosomal gene cluster begins approximately 3.7 kb upstream from the transcription initiation site of the first member of the tandem series.

The rDNA sequence adjacent to the junction (Fig. 2, nucleotides 1 to 138) contains eight poly d(A) tracts (2 to 7 A's in length) interspersed with one to three other nucleotides. This basic pattern is repeated twice (nucleotides 1 to 39 and 54 to 97). This region of rDNA (nucleotides 1 to 97 3′ to the junction) contains more than 60% dA residues, in sharp contrast to the remainder of the ribosomal gene, which tends to be very GC-rich. The poly dA tracts do not continue into the adjacent flanking sequence (Fig. 2, upper tract) nor into the region of C_{BE} that abuts the poly dA-rich segment in the internal repeat units (Fig. 2, lower tract). This latter region of C_{BE} does contain a typical Alu repeat element (*9*) that ends precisely at the junction nucleotide. There are several copies of the Alu repeat in the C region, three of these in C_{BE}. One of these is the one discussed above, and two others occur between the junction and the initiation site. The significance of these sequences remains to be determined.

To verify that the 5′ end of the 15-kb Bam HI clone is derived from distal flanking sequences, and to determine whether or not the sequence is common to all five acrocentric pairs, the 1.6-kb Pst I fragment of λDJ15 (Fig. 1) was used as a probe (henceforth referred to as ACR1) for mapping to a somatic cell hybrid panel and for in situ hybridization to human chromosomes. All cell hybrids containing an acrocentric chromosome scored positive for hybridization to ACR1 while those not containing an acrocentric chromosome tested negative (Table 1). The presence of ACR1 sequence was confirmed on chromosomes 14, 15, 21, and 22, and can be ruled out on chromosomes 3, 4, 5, 6, 7, 10, 12, 16, 17, and X. Furthermore, the hybrid cell line A2 carrying der(X) scored positive whereas the hybrid line B2 carrying der(21) scored negative despite the presence of 40 to 60 copies of rDNA on that chromosome. This confirms that ACR1 is not part of the ribosomal gene repeat unit and that it is specific for sequences distal to the rDNA complex on der(X).

If there is one copy of the ACR1 sequence per acrocentric chromosome, one should expect a hybridization signal corresponding to ten copies per human cell. That this is the case can be seen from Fig. 3A by comparing lanes 1, 2, 5, and 9. Lanes 1 and 2 were loaded with 0.5 μg of human DNA whereas lanes 5 and 9 contain approximately 5 μg of DNA from a hybrid line carrying one acrocentric chromosome per cell. The intensities are similar, consistent with ten copies per cell in lanes 1 and 2. The remaining lanes are not comparable as the amount of DNA per lane was not carefully controlled and the number of acrocentric chromosomes per hybrid cell was variable. The simplest interpretation is that there are ten copies of the ACR1 sequence in human DNA and the sequence distribution on the chromosomes strongly favors a model with one copy of the ACR1 sequence on each of the ten acrocentric chromosomes.

The results with in situ hybridization (Fig. 3, B and C) confirmed these findings as grains were found to be clustered over the tip of all the acrocentric chromosomes. None of the nonacrocentric chromosomes showed a significant cluster of grains, suggesting that the ACR1 probe is specific for the distal region of the acrocentric chromosomes.

Three conclusions can be drawn. First, the rDNA cluster on der(X) is oriented with transcription occurring in a telomere to centromere direction. This is in agreement with the orientation predicted from our previous characterization of the X:21 junction (Fig. 1, XJ) (*6*). Second, the sequences immediately upstream of the rDNA cluster on der(X) appear to be present on all five pairs of acrocentric chromosomes. This conservation of the 5′ end of the rDNA cluster, together with the consistent mapping of the flanking sequence probe ACR1 to the acrocentric short arms strongly suggests that the orientation as well as the sequence are conserved over the five pairs of acrocentric short arms. Third, the rDNA cluster begins approximately 3.7 kb upstream of the transcription initiation site of the first repeat unit, and the consistency of the restriction map strongly suggests that this is a constant feature of the acrocentric chromosomes.

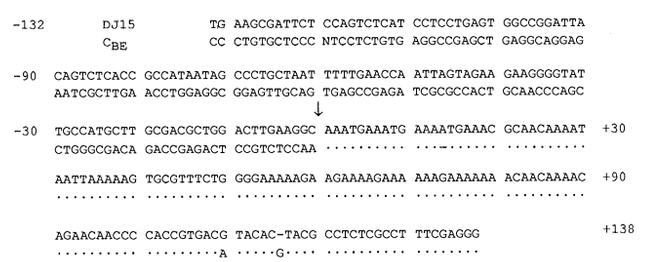

Fig. 2. The comparison of sequences within DJ15 and C_{BE}. Sequencing the 2.9-kb Pst I fragment (Fig. 1B) revealed a region of near identity to C_{BE} and divergence at the 5′ end. The point of divergence is designated the distal junction in λDJ15. The figure gives the sequence of 270 bp surrounding this distal junction. The upper line is that of λDJ15, the lower is C_{BE}; the numbers at the side of the sequence represent the number of nucleotides from the junction. Standard Sanger dideoxy chain termination sequencing was performed on M13 single strand phage recombinants with DJ15- and C_{BE}-derived restriction fragments. The presence of a dot in C_{BE} indicates homology to DJ15. The 5′ Pst I site in λDJ15 is at base pair −155.

Table 1. Hybrid panel mapping of the distal region fragment ACR1.

Hybrid	Acrocentric chromosomes	Nonacrocentric chromosomes	Hybridization signal*
A60-2A	21, 22	2, 4, 5, 7, 17, X	+
W4-3A	14	4, 7, 19, X	+
A23-2E1	13, 14	2, 3, 12, 17, 20, X	+
A48-1G	21	5, 7, 10, 11, 18, 20, X	+
A60-11		5, 10, 12, X	−
t-60-1a		3, 6, 7, 16, 17, X	−
W4-3A-Az cl.2-5		4	−
A60-10	22	4, 12, X	+
A60-8	14	4, 7, 10, 17, 18, X	+
A23-1A cl az31	15	3, 12, 19, X	+
t60-2	14	5, 7, 10, 12, 16, 18, 19, 20, X	+
A60-7	14, 22	3, 4, 6, 11, 16, 17, 20, X	+
FA9-B2	15, 22		+
A2	der(X)		+
B2		3, 5, der(21)	−
AHA-11a		X	−
mA9 (mouse)			−

*Hybridization was determined by digestion of DNA from each hybrid cell line with Pst I, electrophoresis on an agarose gel, and Southern blotting to nitrocellulose filters. Filters were hybridized to the 1.6-kb Pst I fragment, ACR1, that had been radiolabeled with ^{32}P by nick translation. Hybridization was detected by exposure to x-ray film for several hours (Fig. 3). Negative tracks remained negative after prolonged exposure. Isolation and characterization of somatic cell hybrids used in the mapping panel have been described (*21*).

The conservation of the rDNA telomeric junction among the acrocentric chromosomes is of interest in relation to models of chromosome evolution. It has been suggested that the concerted evolution of the rDNA genes relies on homologous recombination between rDNA blocks on nonhomologous acrocentric chromosomes (*10*). This would result in exchange of the distal ends between different acrocentric chromosomes and would, over the course of evolution, result in homogenization of the distal flanking sequences as well as rDNA repeat units (*11*). Our results confirm this prediction. The fact that the more distal "satellite region" of the acrocentric chromosomes is highly variable in size and staining characteristics might well be the result of further recombination events between repeat elements distal to the rDNA that result in a highly variable copy number for the repeat elements. It would be of interest therefore to determine how far into the distal region the sequence homology between acrocentric chromosomes is strictly preserved before running into multiple repeat elements. Probe ACR1 provides a useful starting point for chromosome walking into the more distal region of the acrocentric short arms.

While recombination between ribosomal genes can explain concerted evolution of the ribosomal genes themselves and of the distal sequences on the chromosome, it would not be expected to give rise to concerted evolution of the sequences between the rDNA block and the centromere. Kurnit *et al.* have described a moderately repetitive sequence, pUNC724, that hybridizes preferentially to the pericentromeric region of the acrocentric chromosomes and shows conservation of neighboring restriction sites on these chromosomes (*12*). This suggests that sequences on the proximal side of the ribosomal genes may also be subject to concerted evolution, and if this is the case, the proximal junction (Fig. 1, PJ) of the rDNA cluster should also be conserved over the acrocentric chromosomes. That junction could also be cloned and the hybrid B2 carrying the der(21) would be useful for mapping the junction within the rDNA repeat unit prior to cloning. Studies of the sequences in this proximal region of the acrocentric short arms may shed further light on the mechanisms responsible for the concerted evolution of this chromosomal region.

The ribosomal genes themselves are somewhat variable, displaying polymorphic restriction sites (*13*) and variable numbers of short internal repeat units (*14*). One of the variant patterns previously reported was an 8.5-kb Eco RI fragment extending into the C region from the Eco RI site separating the B and C regions (*15*). The distal flanking region contains an Eco RI site approximately 8.5 kb from the B–C Eco RI site (Fig. 1). This suggests that the 8.5-kb variant clone reported by Mottes *et al.* (*15*) is unlikely to be due to genetic polymorphism, but is probably derived from the distal junction of one of the acrocentric chromosomes. This possible misinterpretation illustrates the considerable difficulty in distinguishing polymorphism from the unique patterns expected at the edges of tandemly repeated blocks of genes.

It is particularly interesting that the 5′ junction of the rDNA cluster should lie a short distance in front of the first transcription initiation site. The region upstream of the initiation site contains the promoter core at nucleotides −45 to +20 (+1 is the initiation site) and an upstream control element at −156 to −107 (*16*). A transcriptional enhancer has also been found in the rat at −1018 to −186 (*17*) while enhancer activity has also been described approximately 2 kb upstream of the transcription start site in yeast (*18*). In principle, the junction at the 5′ end of the ribosomal gene cluster could have been expected anywhere in the first repeat unit since the transcription product of the first gene is presumably not required for cell survival. It is esthetically pleasing, however, to find that the junction is located just upstream from the promoter-enhancer machinery so that the first gene in the block is presumably as functional as the rest. Indeed, it is possible that transcription of the first gene is necessary for transcription to proceed to the remainder of the repeat units as recently suggested (*19*).

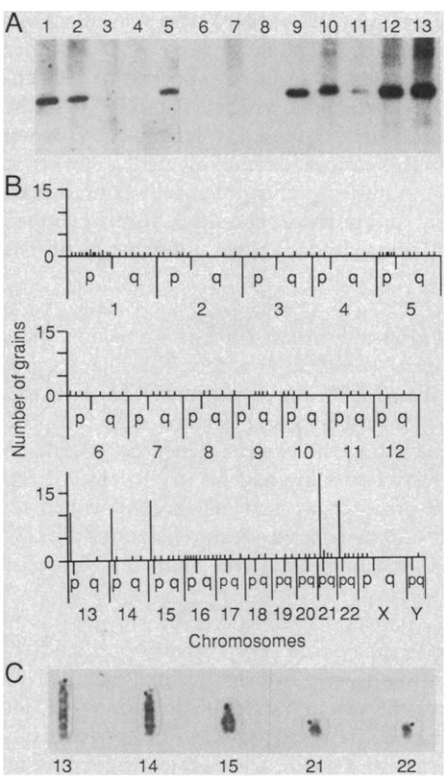

Fig. 3. (**A**) Southern blot analysis with probe ACR1. In each lane genomic DNA was digested with Pst I, separated by electrophoresis on agarose, blotted to nitrocellulose, and hybridized with probe ACR1. The hybridizing band is of size 1.6 kb confirming that the 1.6-kb ACR1 probe is recognizing itself in the genomic DNA. Lanes 1 and 2 contain 0.5 μg of human DNA. Lanes 3 through 13 contain DNA from somatic cell hybrids in the order B2, AHA-11a, A2, A60-11, t60-1a, W4-3A-Azcl2.5, A60-10, A60-8, A23-1AclAz31, t60-2, and A60-7. The human chromosome content of these hybrid lines is provided in Table 1. For convenience, the acrocentric chromosomes present in each lane are given below. The number in parentheses gives the approximate copy number of the acrocentric chromosome per hybrid cell as follows: lanes 3 to 8, no acrocentrics; lane 5, der(X) (1.0); lane 9, 22 (0.9); lane 10, 14 (0.8); lane 11, 15 (0.3); lane 12, 14 (1.0); lane 13, 14 (1.5); and 22 (1.0). Lanes 10 to 13 were loaded with approximately twice as much DNA (10 μg) as lanes 3 to 9. (**B**) Distribution of silver grains on chromosome spreads of human lymphocytes after in situ hybridization with the ACR1 probe. The probe insert was labeled with [^{3}H]thymidine by nick translation and hybridized with metaphase chromosomes as described (*20*). After 11 days of exposure 216 silver grains were scored on 114 metaphase spreads. Accumulations of grains were seen only over the short arms of the acrocentric chromosomes. (**C**) Examples of silver grains over the short arms of each of the five pairs of acrocentric chromosomes.

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8. Clones λDJ15 and λDJ3, were obtained as follows. Human DNA was digested with Bam HI and fractionated by electrophoresis on a 0.6% agarose gel. DNA of size 13 to 17 kb was electroeluted from the gel, purified, treated with calf-intestinal phosphatase, and ligated to the arms of λEMBL-3B [A. M. Frischauf, H. Lehrach, A. Poustka, N. Murray, *J. Mol. Biol.* **170**, 827 (1983)]. The λ vector was prepared by digestion with Bam HI plus Eco RI to release arms with Bam HI ends and a "stuffer fragment" with Eco RI ends. The ligation mix was packaged according to the procedure of Becker *et al.* [A. Becker, M. Marko, M. Gold, *Virology* **78**, 291 (1977)] with noncommercial preparations of proheads, tails, and terminase. Approximately 5 × 10^4

phage were screened by the Benton and Davis procedure [W. D. Benton and R. W. Davis, *Science* **196**, 180 (1977)] with B_{ES} as a ^{32}P-labeled probe. Two positive signals, λDJ3 and λDJ15, were purified through secondary screens and DNA was extracted and purified by standard procedures.
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Risk of Human Immunodeficiency Virus (HIV-1) Infection Among Laboratory Workers

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In a prospective cohort study of 265 laboratory and affiliated workers, one individual with no recognized risk factors for human immunodeficiency virus type 1 (HIV-1) infection was HIV-1 seropositive at the time of entry into the study. Molecular analyses of two HIV-1 isolates derived in two independent laboratories from a blood sample from this worker showed that the isolates were indistinguishable from a genotypic form of HIV-1 present in the H9/HTLV-III$_B$ cell line. Exposure to this strain of virus most probably occurred during work with concentrated virus or culture fluids from virus-producing cell lines under standard Biosafety Level 3 containment. Although no specific incident leading to this infection has been identified, undetected skin contact with virus culture supernatant might have occurred. This worker was the only one found to be positive among the subgroup of 99 workers who shared a work environment involving exposure to concentrated virus. The incidence rate of 0.48 per 100 person-years exposure indicates that prolonged laboratory exposure to concentrated virus is associated with some risk of HIV-1 infection, which is comparable to the risk for health care workers experiencing a needle stick exposure. While none of the ten workers with parenteral exposure to HIV-1 in this cohort became infected, a worker in another laboratory did seroconvert following an injury with a potentially contaminated needle. Strict Biosafety Level 3 containment and practices should be followed when working with concentrated HIV-1 preparations, and further refinement of the procedures may be necessary.

HUMAN IMMUNODEFICIENCY VIRUS type 1 (HIV-1), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), can be transmitted by sexual intercourse, by parenteral inoculation including accidental needle stick, by transfusion of infected blood, or from mother to offspring probably in utero, but not by casual exposure (*1–3*). A few case reports suggest that direct exposure of the skin or mucous membranes to infected blood may result in the development of antibodies to HIV-1 (seroconversion), particularly if the integrity of the skin has been compromised by a dermatologic condition such as eczema (*4*).

For more than 100 years infections have been recognized as occupational hazards among laboratory workers (*5*). Safety guidelines and approaches have been adopted, including the current standards of containment for biologic agents (*6*). Biosafety Level 3 practices are generally recommended for handling concentrated preparations of HIV-1, and Biosafety Level 2 for routine clinical specimens (*7*).

Between 1985 and 1987, invitations to participate in a prospective cohort study to assess laboratory risk were extended to workers from 6 states in 15 laboratory facilities where there was a risk of possible exposure to HIV-1. Each subject signed an informed consent form and completed a two-part questionnaire. Blood samples were analyzed for antibody to HIV-1 and HTLV-I with standard commercially available enzyme-linked immunosorbent assays (ELISA). Follow-up blood samples and questionnaires were obtained every 8 to 12 months. All specimens reactive in ELISA (including those with borderline reactivities) were further evaluated by immunoblots with disrupted HIV-1 and HTLV-I, by radioimmune precipitation assays with labeled HIV-1–producing H9 cells, and by radioimmune assay for specific reactivity to HIV-1 or HTLV-I core (p24) or HIV-1 envelope (gp120) proteins. Consent forms were held by a private physician, an expert in infectious diseases (F.A.G.), who informed each subject about test results. For any subject with a positive or borderline result, the physician provided counseling, performed a standardized physical examination for signs or symptoms of HIV-related conditions, and obtained additional blood specimens for HIV-1 serology and viral isolation (*8*). The first part of the questionnaire focused on occupational exposure to human retroviruses, accidents in the laboratory or elsewhere, and use of biosafety precautions

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