Mapping the *Drosophila* Genome with Yeast Artificial Chromosomes

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The ability to clone large fragments of DNA in yeast artificial chromosomes (YAC's) has created the possibility of obtaining global physical maps of complex genomes. For this application to be feasible, most sequences in complex genomes must be able to be cloned in YAC's, and most clones must be genetically stable and colinear with the genomic sequences from which they originated (that is, not liable to undergo rearrangement). These requirements have been met with a YAC library containing DNA fragments from *Drosophila melanogaster* ranging in size up to several hundred kilobase pairs. Preliminary characterization of the *Drosophila* YAC library was carried out by in situ hybridization of random clones and analysis of clones containing known sequences. The results suggest that most euchromatic sequences can be cloned. The library also contains clones in which the inserted DNA is derived from the centromeric heterochromatin. The locations of 58 clones collectively representing about 8 percent of the euchromatic genome are presented.

STRONG INCENTIVES FOR THE USE OF *Drosophila* AS A MODEL organism for large-scale physical mapping of the genome include its relatively small genome size of approximately 165,000 kilobase pairs (kbp) (*1–3*), the large number of mutations and chromosome rearrangements that are available (*4*), and the ability to carry out germ-line transformation with the use of the transposable element P (*5*). A number of localized regions of the *Drosophila* genome have been analyzed by chromosome walking (*6*), in which sequences obtained from a particular clone are used to identify overlapping clones. Chromosome walks are usually limited to at most a few hundred kilobase pairs, since they require mapping large numbers of overlapping λ or cosmid clones and repeated screening of random libraries of clones. To illustrate the magnitude of the *Drosophila* mapping problem, 10,000 cosmid clones with an average insert size of 33 kbp would be needed for three genome equivalents, which gives a 95 percent probability of including any particular sequence. The physical and genetic analysis necessary to order these fragments and locate them in the genome would be formidable.

The development of yeast artificial chromosome (YAC) vectors (*7*) considerably simplifies the *Drosophila* mapping problem by increasing the size of each cloned fragment. This reduces the number of clones that need to be analyzed and allows the direct identification of overlapping fragments by in situ hybridization to the transverse bands observed in the euchromatic portions of the polytene chromosomes found in salivary glands. The bands serve as reference points for the locations of genes, chromosome puffs, rearrangement breakpoints, and other cytogenetic features, and they have been decisive in correlating *Drosophila* genetic maps with the physical structure of the chromosomes (*8*). The total number of bands in the salivary gland chromosomes has been estimated as between 5059 and 5092 (*9, 10*). Since the euchromatic portion of the genome comprises approximately 110,000 kbp of DNA, the average size of each salivary band is about 22 kbp. Therefore, a YAC containing 220 kbp of *Drosophila* DNA would span approximately ten average salivary gland chromosome bands. Although many of the full complement of bands are very faint and can be visualized only in the most exquisite preparations, even at lower resolution most YAC clones of this size would be expected to cover several major bands, allowing the direct cytogenetic determination of overlaps. In contrast to conventional cloning systems, a *Drosophila* YAC library with three genome equivalents of euchromatic DNA would require only about 1500 clones, reducing the genome mapping problem to a more manageable size.

We describe here the construction and characterization of a YAC library containing large fragments of *Drosophila* DNA. Characterization of the library includes analysis of library representation, the randomness with which the genome was sampled, and the molecular structure of selected clones. Yeast artificial chromosome clones covering localized regions of the genome can be identified by screening the library with probes obtained from conventional vectors or with DNA fragments microdissected from salivary gland chromosomes and amplified by the polymerase chain reaction (PCR) (*11, 12*). To demonstrate the utility of the approach for mapping the *Drosophila* genome on a global scale, we have determined the cytological locations of 58 such clones by in situ hybridization; these clones collectively cover approximately 8 percent of the euchromatic portion of the genome. The ability to localize clones on the salivary map eliminates the requirement for continuity between the clones and makes even a partial map of the *Drosophila* genome useful, since each cloned fragment is assigned to a chromosomal region and immediately becomes available for detailed study.

Library construction and analysis of transformants. The *Drosophila* library of YAC's (DY library) was constructed with a modified YAC cloning vector, pYACP-1 (Fig. 1), that includes the cloning site and a G418 antibiotic–resistance marker (*13*) located within the ends of the *Drosophila* transposable element P (*14*). These

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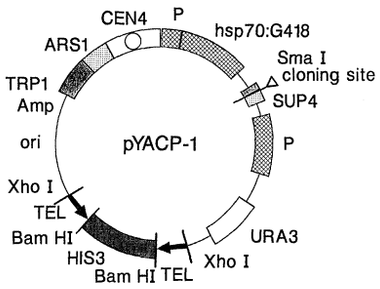

Fig. 1. Yeast artificial chromosome cloning vector pYACP-1. The vector is a modified version of the prototypical vector pYAC2 (*7*). In addition to having the functional elements necessary for the selection and maintenance of clones in *Saccharomyces cerevisiae*, this vector contains the ends of the *Drosophila* transposable element P (*14*) and the *hsp70:G418* resistance gene (*13*). The modifications were carried out as follows. The P-element vector ep19.1 (*45*) was digested to completion with Eco RI and Sal I, and the 1.3-kb fragment containing the 5′ end of the P element, as well as flanking *white* and *rosy* sequences, was subcloned into the plasmid pUC18 to generate the plasmid p18-5P1. This construct was then digested with Eco RI, its ends filled in with the Klenow fragment of DNA polymerase I, and synthetic Sal I linkers were added to generate the plasmid p18-5P2. The resulting plasmid was digested with Sal I to release the 1.3-kb Sal I fragment, and this fragment was cloned into the Sal I site of pYAC2 to generate the plasmid pYAC2-5P. The 3′ end of the P element and the *hsp70:G418* gene were derived from the P transformation vector pUChsneo (*13*). The pUChsneo plasmid was digested to completion with Xmn I and then partially digested with Bal I; the 1.9-kb fragment containing the *hsp70:G418* selectable marker and the 3′ end of P was isolated, and Cla I linkers were added. This fragment was ligated into the Cla I site of a modified pUC18 vector (pUC18C), which had synthetic Cla I linkers ligated in-frame into the Hinc II site of the polylinker, to generate the plasmid p18-3P. The Cla I fragment from p18-3P was then ligated into the Cla I site of pYAC-5P to generate pYACP-1.

modifications allow for the possibility of P element–mediated transformation of the *Drosophila* germ line (*5*) and selection of transformants based on resistance to G418 (*13*). High molecular weight DNA was isolated from the Oregon-R strain of *Drosophila melanogaster* by CsCl gradient centrifugation (*15*). Molecules predominantly larger than 120 kbp were isolated by size fractionation in sucrose gradients (*16*) and were ligated onto vector arms (Fig. 2). Part of this ligation mix was used directly for transformation into *Saccharomyces cerevisiae* and part was fractionated again by size before transformation. Chromosomes from yeast colonies that were expected to contain artificial chromosome clones were prepared from cells embedded in agarose (*17*) and separated by field-inversion gel electrophoresis (FIGE) (*18*). The samples were transferred to nylon membranes (*19*) and hybridized with ^{32}P-labeled pBR322 DNA, which detects sequences present in the pYACP-1 vector arms (*7*) (Fig. 2). DNA blot analysis of 300 transformants from the original ligation mixture showed that approximately 75 percent of the YAC clones were smaller than 50 kbp. Those larger than 50 kbp had an average size of 170 kbp. In contrast, analysis of 382 transformants from the ligation mixture fractionated by size in sucrose showed that approximately 75 percent of the YAC clones were greater than 50 kbp, again with an average size of approximately 170 kbp. A combined sample of 72 clones resulting from transformation with the original ligation mixture and 382 from the fractionated ligation mixture (454 total clones) was used in the subsequent analyses.

To establish the feasibility of the plan for genome mapping, a set of ten *Drosophila*-YAC (DY) clones were initially chosen for in situ hybridizations with salivary gland chromosomes (*20*). After separation from yeast chromosomes by FIGE, the DY chromosomes were excised from the agarose gel, and the DNA was then isolated and used as probe for in situ hybridizations. All ten DY clones hybridized strongly to discrete regions of the salivary gland chromosomes. For example, DY8 is a 300-kbp clone spanning at least eight major salivary bands from the 52B–E region (Fig. 3, A and B). Many additional single-band sites scattered throughout the genome also hybridize with DY8 (Fig. 3A, arrows), but the labeling of these sites is much less intense than the hybridization at 52B–E. The secondary sites of hybridization are due, at least in part, to the presence of repetitive elements within DY8.

To determine the orientation of the DY8 clone relative to the salivary bands and to correlate the physical and cytological boundaries of the cloned DNA, we generated a DNA probe specific for one end of the inserted DNA. This was accomplished with the use of a circular derivative of DY8 (cDY8) originally constructed for transformation experiments mediated by the transposable element P. The cDY8 contains restriction sites that are conveniently located for construction of a plasmid derivative (Fig. 4A). The cDY8 chromosome was generated in vivo by homologous recombination between the linear YAC DY8 and a DNA fragment (introduced by transformation) that contains sequences homologous to both ends of the YAC, as well as genetic markers allowing selection. The recombination event eliminates both telomeres of the artificial chromosome and simultaneously incorporates the *LYS2* gene (*21*) and a nonfunctional *ura3* gene into the newly formed circle. The circular nature of the resulting vector sequences and the preservation of the junctions between vector and inserted DNA were confirmed by DNA hybridization (Fig. 4B). This circularization procedure should be applicable to any YAC chromosome (*22*) and can be used to obtain cloned DNA in a form without free ends (*23*).

A portion of cDY8 containing the bacterial origin of replication (ori) and the ampicillin-resistance gene (Amp^R), as well as sequences derived from the left-hand junction of the inserted *Drosophila* DNA, is included within a Bam HI restriction fragment of the circularized clone (Fig. 4A). When circularized, this fragment forms an autonomously replicating bacterial plasmid. To recover the plasmid (plasmid rescue), DNA from cDY8 was cleaved with Bam HI and circular molecules formed by ligation of the ends; transformation of *Escherichia coli* strain DH5α (*24*) (Bethesda Research Laboratories) and selection for ampicillin resistance yielded the plasmid designated pDY8-L, containing approximately 4.3 kbp of *Drosophila* DNA originating from the left-hand junction between vector and insert in DY8. The plasmid pDY8-L hybridizes to region 52E in salivary gland chromosomes (Fig. 3D). Other sites (not shown) are also labeled, indicating that pDY8-L contains sequences that are repeated in the *Drosophila* genome. The labeling indicates that DNA from the 52E region is closest to the centromere in the YAC.

Analysis of the DY library. The degree to which the DY library is representative of the *Drosophila* genome was assessed by DNA hybridization of selected *Drosophila* DNA fragments to yeast colonies containing the YAC's. We probed 454 yeast colonies containing YAC's with an average size of 170 kbp, which is equivalent to approximately one-half of the genome. If representation is random, the probability that any particular sequence would be included in this set of clones is about 37 percent. Initially, seven single-copy *Drosophila* probes were used to test whether single-copy genes could be detected in the library (*25–31*). Of these probes, four (*white*, *Adh*, *rosy*, *abdA*) identified single DY clones that were subsequently confirmed by DNA blot analysis of FIGE gels with the appropriate probes and by direct in situ hybridization with polytene chromosomes (Fig. 3C). The retrotransposon *copia*, a moderately repetitive sequence typically present in 30 to 40 copies per haploid genome of *D. melanogaster* (*32*), hybridized to 21 YAC-containing colonies; 20 of these were subsequently confirmed by DNA hybridization (Fig. 5A). Since one of the YAC clones found to contain *copia* is DY8, at least some of the secondary hybridization sites found with this clone can be attributed to *copia*. The frequency with which we have isolated clones containing both single-copy and repetitive sequences suggests that there is good representation of the euchromatic portion of *Drosophila* genome in this YAC library.

To assess the randomness with which the *Drosophila* genome is

present in the YAC clones, we examined the *copia*-containing clones by DNA blot analysis (Fig. 5A). Digestion of genomic fragments containing *copia* with Eco RI generates a small (400 bp) internal fragment and two other fragments whose sizes are determined by the location of the nearest Eco RI sites in the flanking genomic DNA (*33*). Since the chromosomal sites occupied by *copia* are highly polymorphic in the Oregon-R strain (*32*), the total number of clonable sites is at least two to three times greater than the number of sites per haploid genome, and few of the cloned *copia*-containing DY clones would be expected to give the same pattern of Eco RI fragments if the genome were randomly sampled for *copia*-containing fragments. When DNA from 18 of the yeast transformants with *copia*-containing DY clones was digested with Eco RI and examined in DNA blots probed with *copia* DNA, none gave the same pattern of Eco RI fragments. This indicates that each *copia*-containing clone is derived from a different location in the genome, which suggests that the DY library is not strongly biased toward cloning DNA from one of these locations over the others.

The internal structures of the DY clones containing *white*, *rosy*, and *Adh* were examined by comparing the lengths of restriction fragments from the DY clones with those in the Oregon-R genome (Fig. 5B). In each case, the bands from the Oregon-R genome are of the expected size, and they match equivalent bands from the corresponding DY clone. A detailed analysis of 160 kbp of cloned DNA derived from the Bithorax complex yielded similar results (*34*). In addition, each of the four YAC clones yielded a single artificial chromosome of the expected size upon repeated subcloning. Taken together, these findings imply that most DY clones contain faithful replicas of DNA sequences present in the *Drosophila* genome and that they are genetically stable in the sense that they do not appear to rearrange at frequencies high enough to be detected during routine subcloning in yeast.

Nevertheless, approximately 1 percent of the DY clones are not genetically stable but rearrange at frequencies high enough that cultures derived from single yeast colonies contain YAC's that are heterogeneous in size as detected by multiple bands in DNA blots of FIGE gels. Many of the unstable clones appear to contain repeated DNA sequences derived from regions of *Drosophila* heterochromatin, such as ribosomal DNA. Among 454 YAC's examined, 6 showed this type of instability on initial testing. Two of these proved to contain *Drosophila* ribosomal DNA. An additional 17 yeast strains with YAC's containing ribosomal DNA were subcloned repeatedly for evidence of instability, and 5 of these showed multiple YAC bands in one or more of the cultures derived from single colonies. Although these results give reason for caution in dealing with YAC's that contain tandemly repeated sequences, the majority of the YAC clones even in this category give no evidence of instability.

Heterochromatin. *Drosophila* heterochromatin occurs primarily in the chromosomal regions flanking the centromeres and consists of a number of kinds of repeated sequences, including ribosomal DNA (located in the X and Y chromosomes), highly repetitive DNA interspersed with unique sequences (β-heterochromatin), and long tracts of highly repetitive, simple sequence DNA (α-heterochromatin) (*35*). Many YAC's in the library appear to contain such sequences. We initially identified DY clones containing sequences that are highly repeated in the *Drosophila* genome by probing DNA blots of FIGE gels with labeled genomic DNA. This procedure identifies only those YAC clones that contain highly repetitive sequences, since these sequences are the most abundant in the probe. Among 32 YAC clones examined in this manner, 5 contained highly repetitive DNA, and 2 of these later proved to contain ribosomal DNA. Furthermore, among 70 YAC clones examined by in situ hybridization, 12 hybridized primarily with the chromocenter, which contains heterochromatin and is much less highly replicated than the euchromatin. Both kinds of evidence suggest that approximately one-sixth of the YAC clones contain some form of highly repetitive DNA. Although the representation, composition, and stability of these clones have not been examined in detail, most of them are stable in the sense that they yield single YAC bands of homogeneous size in cultures derived from single yeast colonies.

Preliminary genome map. We have determined the cytological locations of 58 euchromatic DY clones by in situ hybridization to polytene salivary gland chromosomes (Table 1 and Fig. 6). As expected from the amount of DNA in the DY clones compared to the average amount in a salivary chromosome band, many of the YAC clones encompass multiple salivary bands. In several instances putative cytological overlaps can be identified, for example, among the DY clones in sections 47 and 52 (Fig. 6). Although there appears to be a rough correspondence between insert size and the number of hybridizing polytene bands, clones of similar size may yield very different results depending on their location in the genome. For example, DY493 (260 kb) hybridizes to only one

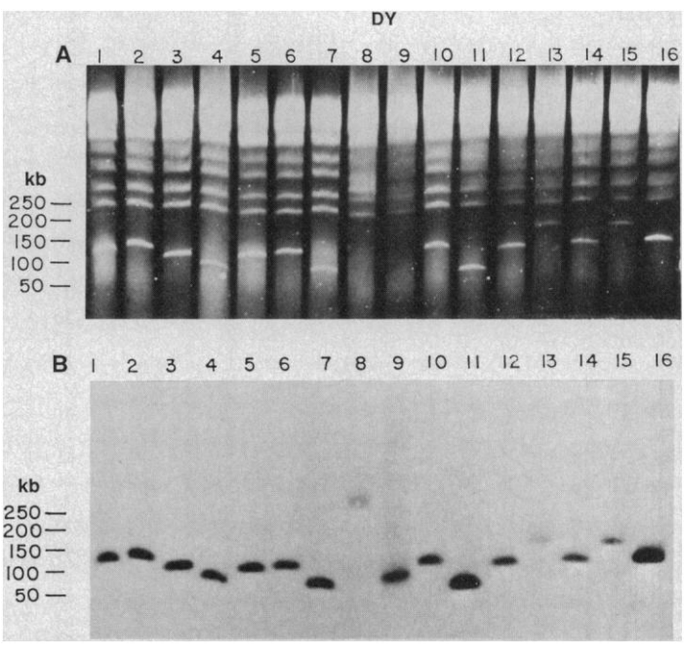

Fig. 2. Analysis of YAC's containing *Drosophila* DNA (DY clones) by FIGE. (**A**) An ethidium bromide (EtBr)–stained gel showing clones DY1 through DY16. Clones were grown in selective media, and chromosomal DNA preparations were made by embedding cells in agarose as described (*17*). Conditions for FIGE (*18*) were 250 V, 12 hours, a forward ramp time of 3 to 60 sec, and a backward ramp time of 1 to 5 sec. (**B**) DNA blot analysis of clones DY1 through DY16. The gel in (A) was blotted (*19*) to a nylon membrane (Hybond-N, Amersham) and hybridized with random hexamer (*46*) ^{32}P-labeled plasmid pBR322. The YAC library was constructed as follows. High molecular weight source DNA was prepared from an Oregon-R strain of *D. melanogaster*. Nuclei were isolated and lysed, and DNA was purified by CsCl gradient centrifugation (*31*). This procedure yielded DNA molecules up to approximately 500 kb in length. The DNA ends were repaired with bacteriophage T4 DNA polymerase (Pharmacia), and fragments were separated by size in sucrose velocity gradients (*47*). Collected fractions were assayed by FIGE, and those fractions containing most of their EtBr-staining material above 120 kb were pooled and concentrated in a collodion bag (Schleicher & Schuell). Size-fractionated DNA was used as insert DNA for ligation with prepared pYACP-1 vector arms, with the use of T4 DNA ligase (Boehringer Mannheim). Part of this ligation mix was fractionated by size and concentrated as above to eliminate small clones and vector arms, and then both the sized and unsized ligation mixtures were used to transform yeast strain AB1380 (*7*). Transformations were done as described (*48*).

major band at 1C, whereas DY20 (170 kb) hybridizes to at least three major bands across 47B–D. These results extend previous results showing that the density of DNA along the polytene chromosomes in the *rosy–Ace* region (87E) varies substantially (*36*). Collectively, the 58 DY clones in Fig. 6 contain 12,000 kbp of DNA, and span an estimated 8 percent of the euchromatic genome of *D. melanogaster*.

Future prospects. Our results demonstrate that representative libraries of YAC clones containing *Drosophila* DNA can be constructed and that DY clones can be localized to specific regions of the *Drosophila* genome by in situ hybridization. Further cytological localizations of DY clones should eventually provide complete coverage of the *Drosophila* genome with YAC clones. The demonstration of overlaps between YAC clones, although in some cases possible cytologically, will more generally require probes that are specific to the ends of the cloned fragments. These probes can also be used for chromosome walking in the YAC library (*11*). A procedure for obtaining end-specific probes from YAC clones by the use of the PCR has been developed (*37–39*), which can substitute for plasmid rescue. Probes from defined regions of the genome can also be generated directly by means of PCR amplification of DNA obtained from microdissected salivary bands (*11, 12*). These probes allow the identification of clones derived from any localized euchromatic region and will be important in filling in gaps in the map, provided the region can be cloned with YAC vectors. As a preliminary test of this method, we have used probe DNA prepared by PCR amplification of DNA microdissected from salivary region 83C–84B and have identified DY clones derived from the region (*11*). If a set of YAC's covering the entire genome were available, any gene localized on the salivary chromosome map would be contained in YAC clones covering the region. The YAC's could also be used as probes for screening cosmid and λ libraries (*40*), allowing direct identification of clones derived from the corresponding regions.

Table 1. Cytological locations of YAC clones.

Location	Size (kb)	DY number
1C	260	493
3C (*white*)	150	430
3F;4A	140	63
4C	140	66
4F;5A	260	524
5E;6C	360	428
11F;12A	200	72
12F;13A	190	62
16F;17A	240	451
17A;17B	330	304
19C;19E	180	24
19E5;19F1–2	280	582
22E;22F	180	666
23D;23F	240	469
24E5;24F6	140	10
26B;26C	250	341
27E;28A	160	13
29E;29F	190	289
29D;29E	150	51
35A;35B (*Adh*)	160	301
36A;36B	280	38
36C;36D	140	30
37A	100	23
41F	260	201
42A	140	55
44F	90	12
47A11;47B4–5	180	19
47B;47D1–2	170	20
47B;47D	240	28
48C;48D1–2	160	314
49F;50A	270	343
52B;52E	300	8
52B1;52C1–2	220	18
52E;53A	250	664
52F;53C	260	506
53C;53D1–3	260	305
53E;53F	290	340
53E4–10	150	11
54E;55A	280	417
58A	120	17
60E;60F4–5	160	48
62D;62E	200	660
66B	300	294
68B;68C	140	672
69A;69C	300	293
72E;72F	150	671
72E;73A	180	70
74E;75A	120	58
75B6;75C	350	187
83E;84B	280	482
87C;87D	160	313
87D (*rosy*)	100	416
89E (*abd-A*)	190	338
92F;93A	240	44
95A;95B	240	54
98E;98F	110	59
99F	240	47
102B	130	35

The use of YAC's may also provide opportunities for the molecular analysis of DNA sequences that cannot be cloned reliably with *Escherichia coli* as the host organism. These include tandemly repeated tracts of simple-sequence DNA, perfect palindromes, long tandem arrays, and miscellaneous, poorly characterized "poison" sequences. Since the *Drosophila* heterochromatin is composed largely of repetitive DNA sequences, this part of the genome has been refractory to molecular analysis. One attempt to walk through a region of β-heterochromatin in region 19E–F at the base of the X chromosome with λ libraries had limited success (*41*). Part of the difficulty in such walks is in the cloning and identification of overlapping clones because of repetitive DNA sequences in the transitional regions between euchromatin and heterochromatin. In contrast, we find that the YAC clone DY582, which hybridizes to salivary section 19E–F (Fig. 6), spans 280 kbp of the transitional region and is apparently genetically stable in yeast. This indicates that some heterochromatic sequences that may be refractory to cloning in *E. coli* can be cloned in YAC libraries. In addition, the ability to clone such repetitive sequences as single, large fragments eliminates many of the problems inherent in attempting to walk through repetitive regions of the genome and raises the possibility of identifying and obtaining sequences associated with centromeres and telomeres, which appear to be embedded in heterochromatin (*42*).

In addition to the apparent advantages for the analysis of the *Drosophila* genome, YAC clones containing large fragments of *Drosophila* DNA offer other experimental possibilities. For example, many genes in *Drosophila* are too large to be cloned as one intact, continuous fragment in conventional cloning vectors, and this limitation restricts the possibilities for manipulation and reintroduction of the genes into the genome. A surprising number of these large genes are involved in the regulation of complex developmental processes, such as the homeotic genes, which control the developmental fates of groups of cells (*43*). A gene for selection in *Drosophila* was included within the ends of the P element in the cloning vector pYACP-1 so that germ-line transformation can be attempted. Prior circularization of the YAC clones (Fig. 4) may be necessary because P element–mediated transformation appears to require circular DNA molecules (*15*). Although the maximum size of molecules that can be transformed is not known, cosmids have been used successfully in transformation (*16*), and transformation with derivatives of

YAC clones should allow at least a modest increase in the maximum size of the integrated DNA. Yeast artificial chromosome derivatives can also be manipulated easily by methods that have been developed for yeast recombination in vivo (*44*). At the moment, the limiting factor in the use of YAC derivatives for transformation is the quantity and purity of YAC DNA that can be obtained.

In this article we have shown the utility of YAC clones in the construction of a global physical map of the *Drosophila* genome. On the other hand, many features of the YAC cloning system itself remain to be investigated, including whether all DNA sequences present in complex genomes can be cloned and why some YAC clones are genetically unstable. The analysis of YAC clones containing *Drosophila* DNA may help resolve some of these issues. In this way the use of YAC's to analyze the *Drosophila* genome will also allow an evaluation of the general utility and limitations of the YAC cloning system.

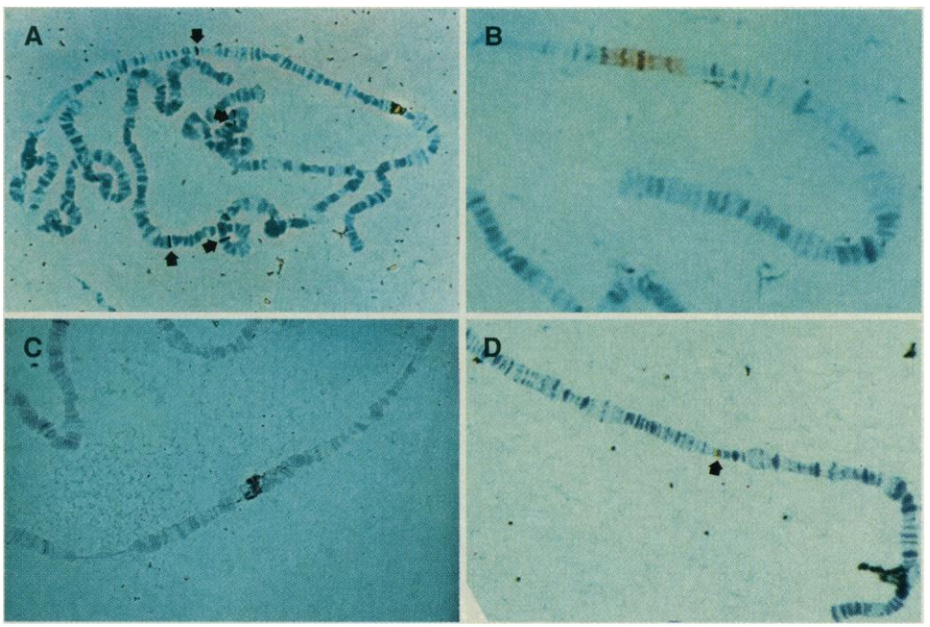

Fig. 3. In situ hybridizations to polytene chromosomes with DY clones. Bands containing individual DY chromosomes were excised from FIGE gels, and the DNA was purified by the use of the NaI–glass powder method (*49*). The DNA was labeled with biotin-dCTP (Enzo Biochemicals) by the random hexamer method (*46*), and in situ hybridizations to polytene chromosome preparations were carried out as described (*20*). (**A**) In situ hybridization with DY8, low magnification. The clone extends cytologically from section 52B to 52E. In addition to the major signal seen at section 52B–E, other faintly labeling sites can be seen (arrows), indicating the presence of repetitive sequences. (**B**) In situ hybridization with DY8, high magnification, showing that the clone labels at least eight major polytene bands. (**C**) In situ hybridization with DY430. This YAC was identified by yeast colony hybridization with a probe from the *Adh* gene (*25*). The YAC hybridizes with cytological position 35B, which is consistent with the known cytological location of *Adh* (*25*). (**D**) In situ hybridization with an end-rescued fragment from DY8. Plasmid recovery of the "left-hand" end (defined as the end of the vector containing the yeast centromere) of cDY8 into *E. coli* strain DH5α (*24*) gave rise to the plasmid pDY8-L (see Fig. 4). Plasmid pDY8-L DNA was isolated (*50, 51*) and used as a probe to determine the cytological orientation of the clone. Since the pDY8-L end probe labels cytological position 52E [compare (B)], the left end of the DY clone is the distal end on the salivary map. Plasmid pDY8-L also contains a repetitive DNA sequence that hybridizes to a subset of the secondary hybridization sites of the entire DY8 clone (not shown).

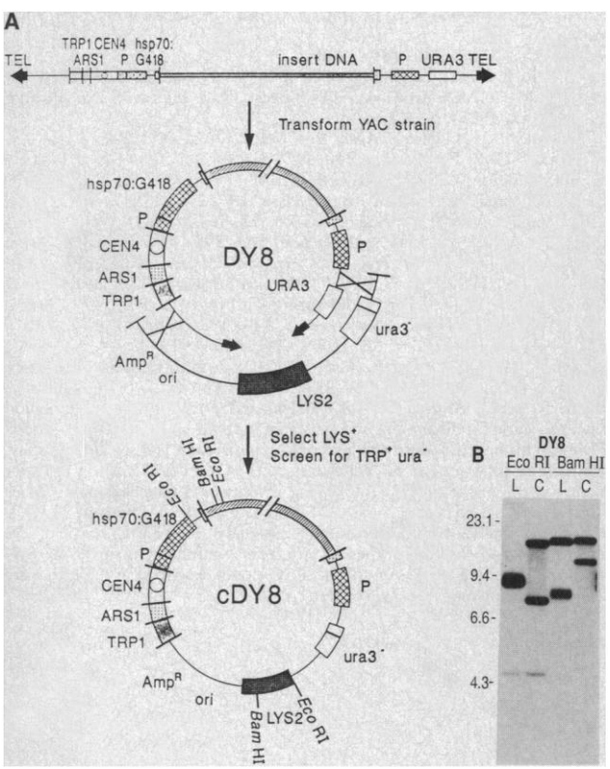

Fig. 4. Circularization of YAC clones by homologous recombination in vivo. (**A**) Diagram of the circularization scheme for DY8. The DY8-bearing yeast strain was transformed with a linear Hind III–Sal I fragment from plasmid pPM680. Homologous recombination between this fragment and the ends of DY8 results in the elimination of the terminal YAC vector sequences and the formation of a circularized DY8 clone (designated cDY8). Transformants were selected for lysine prototrophy and subsequently screened for uracil auxotrophy. The plasmid pPM680 was constructed as follows. Plasmid YIP5 (*52*) was digested to completion with Nco I, treated with the Klenow fragment of DNA polymerase I, and religated to create plasmid pPM661; this introduces a frameshift mutation into the *URA3* gene. The *LYS2* gene (*21*) was isolated as an Eco RI–Hind III fragment, the ends were filled in with Klenow, and the fragment was ligated into the Pvu II site of pPM661 to create plasmid pPM680. (**B**) DNA blot analysis of cDY8 circles. Yeast DNA was prepared (*53*) from the linear (L) and circular (C) DY8-bearing strains, digested with either Eco RI or Bam HI, fractionated in an 0.8 percent agarose gel, and blotted to a nylon membrane (Amersham). Hybridizations were carried out with ^{32}P-labeled pBR322 as the probe. An Eco RI fragment of approximately 9 kb is diagnostic for all linear DY chromosomes derived from pYACP-1, whereas a much larger fragment of 12.5 kb is generated from the circularized derivatives of these clones. The relative sizes of Bam HI fragments also differ, with the left arm of the linear fragment slightly smaller than the analogous Bam HI fragment from the circularized version, and the right hand of the linear fragment approximately 3 kb smaller than the fragment generated from the circle. Minor variation is expected in the sizes of the terminal fragments because of heterogeneity in the lengths of the added yeast telomeres (*50*). Plasmid recovery was carried out on cDY8 (size approximately 13.5 kb) as follows. Approximately 10 μg of DNA from a cDY8-bearing yeast strain was isolated, digested with Bam HI, extracted once with phenol, once with a mixture of chloroform and isoamyl alcohol (24:1), and precipitated with ethanol. The DNA was resuspended in TE (10 m*M* tris-HCl and 1 m*M* EDTA, *p*H 8) and self-ligation was carried out in a final volume of 1 ml (approximately 10 μg/ml) to promote the formation of intramolecular reaction products. Ligation mixtures were precipitated with ethanol, resuspended in TE, and used to transform competent bacterial cells from strain DH5α (*24*).

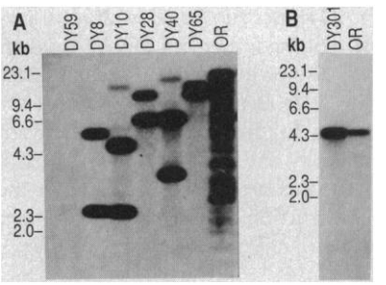

Fig. 5. DNA blot analysis of DY clones containing *copia* and *Adh*. Clones were originally identified by colony hybridization with either (**A**) *copia* or (**B**) *Adh* probes (*25, 33*). DNA was prepared, digested with Eco RI, subjected to electrophoresis in 1.0 percent agarose, transferred to a nylon membrane (Amersham), and hybridized with either the (A) *copia* or the (B) *Adh* probes. These blots contain approximately 10 μg of Oregon R and 1 μg of yeast DNA per lane. (A) Each clone containing *copia* is expected to yield two junction fragments that begin at an internal Eco RI site and extend to the closest genomic Eco RI site flanking the particular *copia* element. In this experiment each of 18 DY clones containing *copia* yields a different pattern of Eco RI junction fragments, indicating that the *copia* elements are associated with a different fragment of genomic DNA. Five such clones are shown as well as the Oregon-R strain used as the DNA source for the library. DY59 contains no *copia* element and is included as a negative control. (B) Comparison of Eco RI fragments from Oregon R and DY301 that are homologous to an *Adh* probe. Both fragments are of the size predicted from the cloned *Adh* gene (*25*).

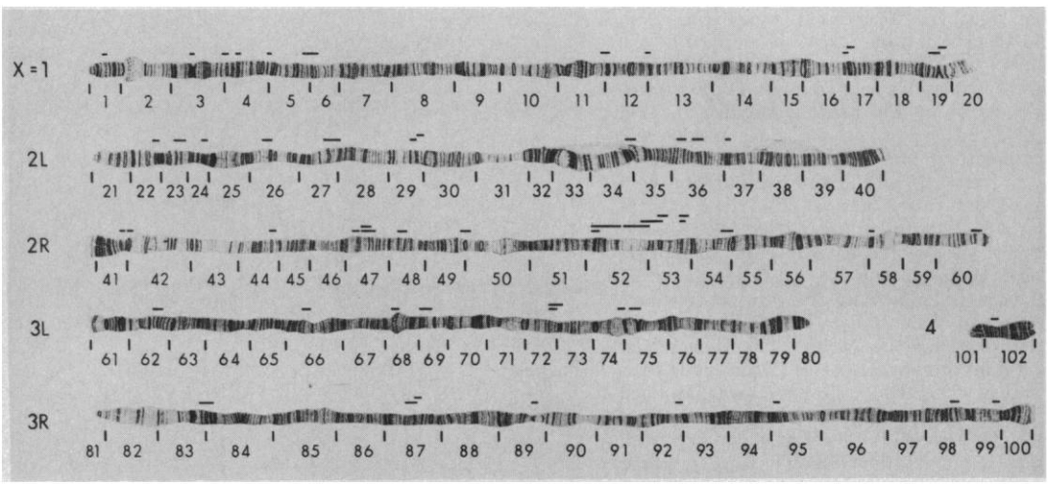

Fig. 6. Localization of 58 DY clones on the salivary polytene chromosome map (*4*). Each clone is shown as a solid line above the salivary map. The positions of the boundaries of the clones are approximate (see Table 1). Single colonies were isolated and cultured from the DY-bearing yeast strains, and the YAC DNA was prepared and labeled as described in Fig. 3. The average size of the clones used in these hybridizations was approximately 200 kbp. In many cases multiple salivary bands are labeled, and overlaps can often be identified cytologically, for example, in sections 47 and 52.

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22. We have carried out circularization of seven DY clones ranging in size from 70 to 300 kb. While such circles migrate anomalously on FIGE gels, the relative migration of circles of different sizes can be used to identify primary circularization events. However, we have not shown directly that such clones contain the same DNA insert as in the linear DY from which they are derived, and rearrangement events during circularization may occur.
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