Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2698–2702, May 1985
Biochemistry

Cloning of *Physarum* actin sequences in an exonuclease-deficient bacterial host

(inverted repeat/instability of recombinant DNA/replacement vector EMBL3/*recBC* gene/*sbcB* gene)

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Communicated by Franklin W. Stahl, January 2, 1985

ABSTRACT **A genomic library of *Physarum* was constructed in the replacement vector EMBL3. Efficient propagation of the recombinant phages occurred only on the *recBC*$^-$ *sbcB*$^-$ host *Escherichia coli* CES200, which is deficient in the exonucleases I and V. Thirteen different recombinants with actin-related sequences were detected and 10 were purified from 90,000 plaques (the equivalent of 6 *Physarum* genomes) on strain CES200. Comparison of the plating efficiencies of the library and the actin-related isolates suggests that palindromic DNA sequences are responsible for the instability of *Physarum* DNA in *E. coli*. In one of these isolates, λPpA10, and in a 2.81-kilobase subclone of that isolate in plasmid pBR322, a deletion of 360 base pairs was detected that led to stable propagation of the recombinant DNA molecules in Rec$^+$ *E. coli*. Electron microscopic analysis of the 2.81-kilobase fragment, after denaturation and self-hybridization, revealed secondary structures consistent with "foldback" structures. Restriction and DNA blot analysis of λPpA10 suggest that the unstable DNA segment is in close proximity to, if not part of, the previously defined actin-gene locus *ardA*.**

Construction and use of libraries of genomic DNA in plasmid, cosmid, or phage λ vectors are standard methods and important tools for the study of gene structure and function (1). Application of recombinant DNA techniques to the plasmodial slime mold *Physarum polycephalum* would be very useful because *Physarum* is a model organism for the investigation of gene replication during the eukaryotic mitotic cycle and cellular differentiation (2). The actin gene family of this organism, for example, has been characterized by Mendelian segregation of polymorphic restriction fragments (3), and the order of replication of these genes has recently been determined in our laboratory (4). Thus, it is surprising that up to now only one gene, a putative histone H4, has been isolated from a genomic library (5). Problems with maintaining recombinant phages and with the isolation of *Physarum* genes, for example those for actin and tubulin, have been reported by different groups (ref. 6; ||). Instability of certain eukaryotic DNA sequences in *Escherichia coli* has been demonstrated in response to the prokaryotic recombination system (7, 8). The stability of palindromic structures, artificially constructed in plasmid (9) and λ DNA (10), has also been studied. It has been found that propagation of these sequences is permitted only in bacterial hosts lacking both exonucleases I and V, the products of the *sbcB* and *recBC* genes, respectively (for reviews, see refs. 11 and 12). It has been shown by electron microscopy (13, 14) that in single-stranded *Physarum* DNA, "foldback" structures can be observed after intramolecular reassociation; these structures are regularly spaced throughout the genome. Here we present evidence that palindromic structures in *Physarum* DNA may cause low plating efficiencies of a genomic *Physarum* library in the λ replacement vector EMBL3 on Rec$^+$ *E. coli* hosts and that these structures also may cause instability of actin-related sequences obtained from this library that are cloned either in phage λ or the plasmid pBR322.

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MATERIALS AND METHODS

Construction of the Genomic Library. DNA was isolated from nuclei of axenically grown *Physarum* amoebae, strain Cld-Axe (15). The nuclei were lysed in 50 mM Tris Cl, pH 8/0.1 M EDTA/proteinase K (100 μg/ml)/0.5% *N*-lauroylsarcosine for 1 hr at room temperature. High molecular weight DNA was obtained by phenol extraction, RNase treatment, and extensive dialysis against 10 mM Tris Cl, pH 8/1 mM EDTA (16). Electrophoresis in a 0.4% agarose gel revealed a major DNA band at ≈100 kilobases (kb) and a minor band at 60 kb, which probably represents the intact linear, extrachromosomal ribosomal DNA of *Physarum*. The DNA was partially digested with restriction endonuclease *Mbo* I, and 15- to 20-kb fragments were isolated from a sucrose gradient (1). DNA from bacteriophage EMBL3 was digested with *Bam*HI and *Eco*RI, and the small linker fragment was removed by isopropanol-precipitation (17). The *Physarum* DNA fragments were then ligated to the *Bam*HI sites of the phage arms in a 1:4 molar ratio. The ligated DNA was packaged *in vitro* according to Mullins *et al.* (18) or with an *in vitro* packaging kit (Amersham).

Isolation and Characterization of Bacteriophage and Plasmid DNA. Bacteriophages were prepared from cultures of infected *E. coli* strains LE392 and CES200 in L-broth, and DNA was isolated as described (19, 20). DNA from recombinant pBR322 plasmids was introduced into *E. coli* by transformation by the calcium chloride method (21). For preparation of plasmid DNA, the cells were lysed by boiling (22) and the lysate was extracted once with phenol and then with chloroform immediately after centrifugation to remove denatured protein and chromosomal DNA. The plasmid DNA was further purified on isopycnic CsCl gradients containing ethidium bromide. DNA labeling by nick-translation (specific activity 10^8 cpm/μg of DNA) and restriction mapping by multiple digestion were done as described (23, 24). For DNA blotting and hybridization we followed the procedure of Southern (25) with modifications (3, 4). The probe was a fragment of *Strongylocentrotus purpuratus* actin-encoding genomic DNA, which was prepared from plasmid pSpG17 by preparative gel electrophoresis after

Abbreviation: kb, kilobase(s).

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||Cox, R. A., Hardman, N., Laffler, T. G., Schreckenbach, T. & Wilhelm, F.-X., Sixth European *Physarum* Meeting, April 24–28, 1984, Font-Romen, France.

Table 1. Relative plating efficiencies of the *Physarum* library and of selected phages with actin-related sequences on different *E. coli* hosts

E. coli strain	Relevant genotype	Relative plating efficiency, %					
		EMBL3	Library	λPpA10	λPpA10′	λPpA3	λPpA3′
CES200	*sbcB15 recB21 recC22 hsdR*$^-$	100	100	100	100	100	100
LE392	*hsdR514*	114	17	15	110	16	129
NM519	*sbcA23 recB21 recC22 hsdR24*	101	21	11	119	6	140
SK1592	*sbcB15 hsdR4*	90	6	5	105	9	100
Q359	*hsdR*$^-$ P2	0	8	ND	ND	ND	ND

Cultures were grown to stationary phase in L broth plus 0.2% maltose, spun down, and resuspended in 10 mM Tris Cl, pH 8/10 mM $MgSO_4$. All platings were done on L-broth plates with 10 mM $MgSO_4$ at 37°C. With the exception of strain Q359, the plating efficiency of each culture for *in vitro* packaged undigested EMBL3 DNA was 0.8–1 × 10^8 plaque-forming units per μg of DNA and each relative plating efficiency is a mean value of three independent experiments. Efficiencies on strain CES200 are set equal to 100%. The titer of EMBL3 DNA was 9 ± 1 × 10^7 plaque-forming units per μg; that of the library was 1.2 ± 0.4 × 10^6 plaques per μg of *Physarum* DNA on strain CES200 after subtraction of 18% library background. For the other hosts, the plating efficiency of the library was corrected by subtracting the individually determined background. This indicates that approximately only 20% of the *Physarum* genome can be cloned in common Rec$^+$ host strains. The phages λPpA10 and λPpA3 were derived from plaques after plating on strain CES200, whereas λPpA10′ and λPpA3′ were from plaques after plating on strain LE392. ND, not done.

digestion with endonuclease *Hin*dIII (26) and shown to be specific for actin-related sequences of *Physarum* (4).

For electron microscopy, recombinant plasmid DNA was digested with *Eco*RI and *Hin*dIII, and after electrophoresis in 0.6% agarose, the 2.81-kb *Physarum* insert was isolated from the gel by the "freeze-squeeze" method (27). Eluted DNA was denatured in 35% formamide at 70°C for 5 min and allowed to self-hybridize at room temperature for 30 min with or without 125 mM ammonium acetate. The DNA was spread onto a 10% formamide hypophase, picked up on collodion-coated grids, and examined by electron microscopy as described (28).

RESULTS

Construction of a Genomic Library of *P. polycephalum*. A genomic library of 15- to 20-kb DNA fragments from *Physarum* amoebae (strain Cld-Axe) (15) was constructed in the λ replacement vector EMBL3 (17). Several different host strains were infected with the recombinant phages (Table 1). Except for the P2 lysogen Q359, the efficiency of plating of *in vitro* packaged wild-type EMBL3 DNA was 0.8–1.0 × 10^8 plaque-forming units/μg of DNA. Digested vector DNA, religated in the absence of *Physarum* DNA, was also packaged *in vitro* and the plating efficiency of those phages was considered as background. As can be seen from Table 1, the highest plating efficiency was achieved with the *recBC*$^-$ *sbcB*$^-$ host strain CES200, which is deficient in exonucleases I and V. In several experiments, 1 μg of *Physarum* DNA yielded ≈10^6 recombinant phages on stain CES200. This value has been corrected for background (15–20%) and has been set as 100% in Table 1. The library yield dropped under 25% when the *Physarum* library was plated on the isogeneic *recBC*$^-$ *sbcA*$^-$ host NM519 [a *hsdR*$^-$ derivative of strain JC8679 (29)], which has exonucleases I and VIII but not exonuclease V, and on the non-isogeneic strains LE392 and SK1592. LE392 is a Rec$^+$ host, derived from strain ED8654 (30); SK1592 (21) is a *sbcB*$^-$ mutant, thus lacking exonuclease I but not V. Poor efficiency was also observed with Q359 (31), a P2 lysogen, thus precluding positive selection for Spi$^-$ EMBL3 recombinants.

Isolation of Phages with Actin-Related Sequences from *Physarum*. Approximately 90,000 nitrocellulose-filter replicas of bacteriophage plaques from the *Physarum* genomic library were screened with the radioactively labeled probe for actin-related sequences. Lanes 1 in Fig. 1 *b* and *d* show the hybridization signals of *ardA*, *-B*, *-C*, and *-D* after digestion of nuclear DNA with restriction endonucleases *Eco*RI or *Hin*dIII. We detected 13 plaques with actin-related sequences when the library was plated on strain CES200 and none when 90,000 recombinant clones were plated on host strains LE392 or Q359. Of the 13 putative actin clones, 10 were isolated and 3 were lost during further plaque-purification. DNA of 6 recombinant phages was isolated and analyzed after digestion with *Eco*RI and *Hin*dIII by DNA blotting (Fig. 1). The *Eco*RI fragment of λPpA10 (Fig. 1*d*, lane 6, and Fig. 2*b*) has the same size as the corresponding restriction fragment in genomic DNA, defined as *ardA*. The restriction patterns of λPpA10 (lane 6) show a minor *Eco*RI band of 8.8 kb, smaller than the typical *ardA Eco*RI fragment of 9.2 kb This indicates instability of an actin-related se-

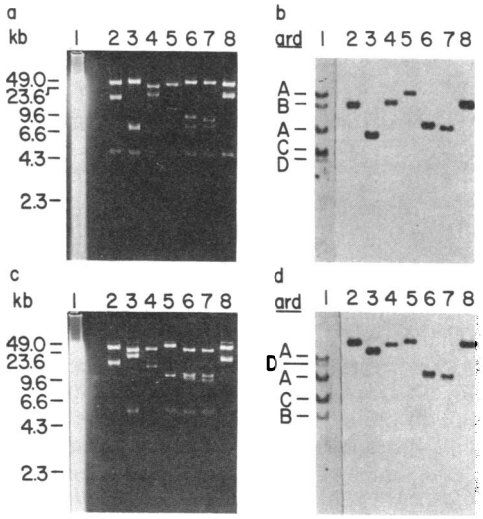

FIG. 1. Hybridization of DNA from *Physarum* and recombinant EMBL3 phages with actin-gene probe. DNA (10 μg) from *Physarum* Cld-Axe amoebae (lanes 1) and 1 μg of DNA from recombinant phages λPpA1, -2, -3, -4, -10, -10′, and -12 (lanes 2 through 8, respectively) were digested with *Hin*dIII (*a* and *b*) or *Eco*RI (*c* and *d*) and size-fractionated in 0.6% agarose gels. The gels were stained with ethidium bromide (*a* and *c*) and, after denaturation, the DNA was blotted onto nitrocellulose filters. The DNA blots were hybridized with a nick-translated sea urchin actin-gene probe, washed, and autoradiographed (*b* and *d*). The bands in lanes 1 (*b* and *d*) are designated as *ardA*, *-B*, *-C*, and *-D* as defined previously (3); size markers were fragments of *Hin*dIII-digested λ DNA.

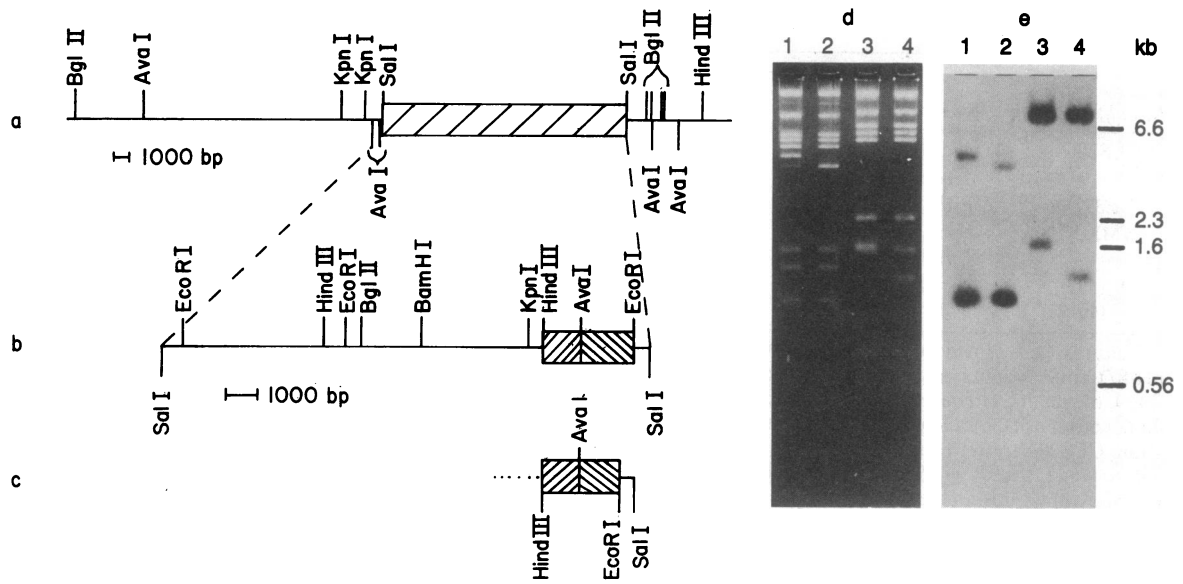

FIG. 2. Restriction map of λPpA10 and hybridization of its DNA. (*a–c*) The restriction map was constructed by multiple digestion (24). The restriction sites on the vector DNA (*a*) and the sites on the inserted *Physarum* DNA (*b*) are indicated. The hatched blocks between the *Eco*RI and *Hin*dIII sites in *b* and *c* indicate the location of the actin-related sequence and the site where frequent deletion occurs. The segment in *c* was derived from λPpA10′ DNA. bp, Base pairs. (*d*) Ethidium bromide-stained 1.4% agarose gel after electrophoresis of 1-μg samples of λPpA10 (lanes 1 and 3) and λPpA10′ DNA (lanes 2 and 4), digested with *Hin*dIII and *Ava* I (lanes 1 and 2) or *Ava* I and *Eco*RI (lanes 3 and 4). λPpA10′ is a progeny phage from a plaque formed by λPpA10 after plating on strain LE392. (*e*) Autoradiograph of the DNA blot of the gel in *d* after hybridization with the nick-translated actin-gene probe.

quence in recombinant phages. The other 5 clones do not contain the complete *Hin*dIII or *Eco*RI restriction fragments of the *ard* loci.

Instability of the Isolated Phages in Different *E. coli* Hosts. Aliquots of the six phages with actin-related sequences were plated on the hosts CES200 and LE392. In each case, the plating efficiency on the Rec$^+$ host was approximately one-seventh that on the *recBC*$^-$ *sbcB*$^-$ strain. A similar difference was observed when phages λPpA3 and λPpA10 were plated on *sbcB*$^-$ and *recBC*$^-$ *sbcA*$^-$ hosts (Table 1). However, the phages obtained by replating λPpA3 and λPpA10 on Rec$^+$ host LE392 revealed the same plating efficiency as that observed with the *recBC*$^-$ *sbcB*$^-$ host (Table 1). DNA from one of the progeny phages of λPpA10 was analyzed on DNA blots (Fig. 1, lane 7). Its *Eco*RI and *Hin*dIII fragments with actin-related sequences had the same size as those fragments observed as minor components of λPpA10 DNA from strain CES200 (see above). We conclude that this recombinant phage became stable after a small segment of the *ardA* sequence was deleted. Restriction maps of the deleted and undeleted λPpA10 were constructed (Fig. 2 *a–c*). The deletion was found in a 1.71-kb *Ava* I–*Eco*RI fragment that hybridized weakly with the sea urchin actin probe (Fig. 2*e*). A much stronger hybridization signal was obtained with the adjacent 1.1-kb *Hin*dIII–*Ava* I fragment. Once again, the smaller *Ava* I–*Eco*RI fragment was found as a minor component of λPpA10 DNA after plating on strain CES200 as host (Fig. 2*d*).

Subcloning in pBR322. The 2.81-kb *Hin*dIII–*Eco*RI fragment from λPpA10 was isolated by preparative gel electrophoresis, ligated to the *Hin*dIII–*Eco*RI sites of pBR322 and both the *recBC*$^-$ *sbcB*$^-$ strain CES200 and the *recA*$^-$ strain HB101 were transformed. As can be seen from Fig. 3*b*, the inserted DNA is unstable in both strains and becomes shortened to give the 2.45-kb fragment observed in the recombinant λ phages. The intensity of the bands indicates that deletions occur in HB101 much more frequently than in CES200. During purification of plasmid from CES200, we found DNA yields about 5% those for HB101, which is probably due to the reported instability of pBR322 in *recBC*$^-$ *sbcB*$^-$ *E. coli* bacteria (32). A restriction map was constructed for the complete (2.81-kb) and deleted (2.45-kb) inserted DNAs by double digestions of the corresponding plasmids, first with *Hha* I, *Sau*3A, or *Hae* III and then with *Eco*RI or *Ava* I. As is indicated in Fig. 3*a*, the deletion removes three *Hha* I sites and one *Sau*3A site. *Hha* I digests of the plasmid with the deleted fragment showed a 1.64-kb fragment instead of the four fragments of 0.62, 0.04, 0.30, and 1.04 kb found for the undeleted plasmid. A second digest with *Ava* I and *Eco*RI revealed an *Ava* I restriction site in the 0.62-kb and an *Eco*RI site in the 1.04-kb undeleted *Hha* I fragments, whereas the deleted 1.64-kb segment was cut only once by both enzymes. The deletion breakpoints in the

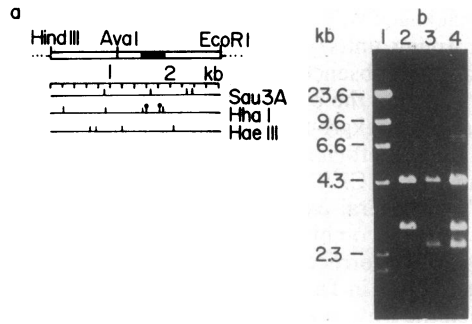

FIG. 3. Restriction map of the *Hin*dIII–*Eco*RI fragment of λPpA10, subcloned in plasmid pBR322. (*a*) For the map construction, plasmids with the deleted and undeleted *Hin*dIII–*Eco*RI fragment were digested first with *Hin*dIII, *Ava* I, or *Eco*RI and then with *Sau*3A, *Hha* I, or *Hae* III. The digests were electrophoresed in 2% agarose or 5% or 20% polyacrylamide gels, and restriction-fragment patterns were compared with those from single digests. The solid bar indicates the deletion; one *Hha* I site could not be definitively determined (• •). (*b*) Lanes 2–4: Plasmid DNA was electrophoresed in a 0.9% agarose gel after digestion with *Eco*RI and *Hin*dIII. The DNA was derived either from ampicillin-resistant CES200 (lane 2) or HB101 (lane 4) isolates after transformation with pBR322 recombined with the undeleted λPpA10 *Hin*dIII–*Eco*RI fragment or from a CES200 isolate (lane 3) transformed with pBR322 containing the deleted fragment. Lane 1: *Hin*dIII-digested λ DNA.

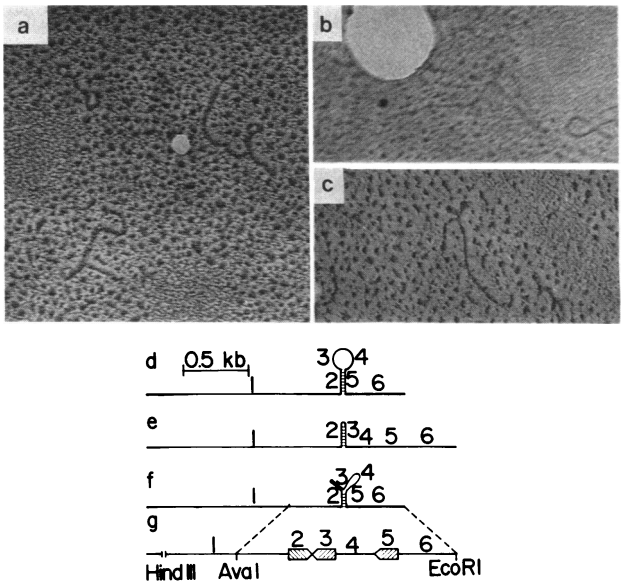

FIG. 4. Secondary structures in the *Hin*dIII–*Eco*RI fragment of λPpA10. (*a–c*) Micrographs of the full-length single-stranded and self-hybridized 2810-nucleotides fragment subcloned in and purified from pBR322. The two structures in *a* were interpreted as two looped hairpins, whereas *b* shows an unlooped hairpin and *c* shows a Y-shaped structure. (×30,240.) (*d–f*) Interpretative drawings of the observed looped and unlooped hairpins. For size determination we measured 30 molecules, self-hybridized in 0.125 M ammonium acetate. No differential scaling was used for single- and double-stranded regions because it is unclear how many base pairs actually establish the foldback structure. The 16 structures that were larger than average size were considered as full-length molecules and their length of 2.81 kb, determined in agarose gels, was used as an interval size standard. (*d*) The stem (**2,5**) measured 190 ± 20 nucleotides, the loop (**3,4**) was 580 ± 100 nucleotides, and the flanking sequences (**1** and **6**) were 1470 ± 50 and 520 ± 40 nucleotides long. (*e*) Hairpins (**2,3**) measured 190 ± 10 nucleotides, and the flanking sequences, (**1**) 1530 ± 80 and (**4,5,6**) 880 ± 70 nucleotides. (*f*) The first stem (**2,3**) measured 70–170 nucleotides, the second stem (**2,5**) measured ≈100 nucleotides or was absent, the loop (**3,4,5**) measured 490 ± 30, and the flanking sequences (**1** and **6**), 1470 ± 36 and 450 ± 20 nucleotides, respectively. (*g*) A model to explain the foldback structures (see text). The data for *d–f* were derived from measurements of 7, 4, and 5 molecules, respectively.

2.81-kb *Hin*dIII–*Eco*RI fragment are flanked by a 1.53-kb DNA stretch of the actin-related sequence and by 0.92 kb of DNA toward the *Eco*RI site.

Secondary Structures in the Unstable Actin Sequence of *Physarum* DNA. The *Hin*dIII–*Eco*RI fragment of *ardA* from λPpA10 was isolated from the plasmid, denatured, and allowed to self-hybridize in the absence or presence of 125 mM ammonium acetate. Three characteristic intramolecular secondary structures were detected under the electron microscope in 30 out of 35 molecules self-hybridized in the presence of the salt and in 13 out of 63 molecules self-hybridized in its absence. Under both conditions, the most frequent structure was a stem and loop, observed in 60% of the molecules with secondary structures (Fig. 4*a*); hairpins (Fig. 4*b*) and a Y-shaped structure (Fig. 4*c*) each occurred with a frequency of 20%. In each case, the secondary structure is flanked on one side by a sequence of 1500 nucleotides. On the other end, the molecules with hairpins contain an ≈880-nucleotide DNA sequence which is reduced to ≈500 nucleotides in molecules with stem-loops and hairpins with loops (Fig. 4 *d–f*). Hairpins and stems were ≈190 nucleotides long, containing 380 total nucleotides. Our interpretations of the intrastrand base-pairing responsible for the stem and loop, hairpin, and Y-shaped structures are shown in Fig. 4 *d*, *e*, and *f*, respectively.

DISCUSSION

We have shown that the majority of recombinant phages in a library of genomic *Physarum* DNA could form plaques only on a *recBC*$^-$ *sbcB*$^-$ host, CES200. This strain was prepared by C. Shurvinton in the laboratory of F. Stahl from strain JC9387, a *sup*$^-$ derivative of JC7623 (33). With this strain, an efficient screening for recombinant DNA molecules with actin-related sequences became possible. From 90,000 plaques [representing ≈6 *Physarum* genomes (34)], we detected 13 different phages with DNA homologous to actin-encoding sequences. Ten clones were plaque-purified, and six were analyzed by restriction mapping; one clone, λPpA10, contained a complete restriction fragment previously identified as actin locus *ardA* (3). The apparent contradiction, that no actin-related λ recombinants can be detected on Rec$^+$ hosts but that 15% of all recombinants isolated from the EMBL3/CES200 library could be maintained on the Rec$^+$ strain, might be explained by deletions, which probably occurred during plaque-purification on host CES200. These deletions might eliminate the proposed palindromic structures. The actin-related sequence of λPpA10 was subcloned in plasmid pBR322, propagated in strain CES200, and partially characterized. From our results for plating frequencies, deletion mapping, and electron microscopy, we suggest that the instability of *Physarum* DNA is largely due to palindromic sequences. Efficient plating of the library and of the isolated recombinant phages and plasmids was only possible on a host without exonuclease I and V activities (*sbcB*$^-$ *recBC*$^-$) but not on hosts lacking only one of the enzymes (Table 1). The latter result excludes the possibility that instability and deletion are caused by bacterial *recE* recombination (11, 12), as observed with a sequence from the kangaroo rat (8). Further evidence for instability through inverted repeats comes from the analysis of one of the isolated recombinants. In this phage, λPpA10, a sequence of 360 nucleotides was deleted at a low frequency in the *recBC*$^-$ *sbcB*$^-$ strain and was totally lost in a Rec$^+$ host. Leach and Stahl (10) observed similar excision frequencies of their *in vitro*-constructed palindrome in phage λ after plating on Rec$^+$ and *recBC*$^-$ *sbcB*$^-$ host bacteria. When the 2.81-kb fragment of λPpA10, which contains the unstable sequence, was subcloned in the plasmid pBR322, a deletion was observed in the same region on a *recA*$^-$ host, again with a much higher frequency than in the *recBC*$^-$ *sbcB*$^-$ strain. Similar excision rates for palindromes in plasmids were observed by Collins *et al.* (9). Lower plasmid yields from strain CES200 might be explained by the reported instability of pBR322 in *recBC*$^-$ *sbcB*$^-$ bacteria and might be overcome by introduction of either *recA* or *recF* mutations into the *recBC*$^-$ *sbcB*$^-$ background (32).

The cloned 2.81-kb *Eco*RI–*Hin*dIII fragment revealed specific secondary structures after denaturation and self-hybridization when visualized under the electron microscope. The unlooped hairpins measured ≈380 nucleotides total length and were flanked by ≈1500 and 900 nucleotides. Assuming that the long flanking sequence terminates at the *Hin*dIII site and the short sequence, at the *Eco*RI site, these measurements match the dimensions of the deletion breakpoints determined by restriction mapping and suggest that the deleted sequence is a foldback structure. DNA sequencing is necessary to test this hypothesis and to determine how many base pairings contribute to the foldback structures.

Another inverted repeat, designated **5** in Fig. 4 *d–g*, might occur 0.3 kb from the first repeat toward the *Eco*RI site and could explain the folding of the molecule to form looped hairpins and Y-shaped structures (Fig. 4*g*). We propose from the cloning requirements, frequent deletion, and electron microscopy that the instability of recombinant *Physarum*

DNA upon propagation in certain bacterial hosts is caused by certain foldback DNA sequences. Furthermore, propagation of other recombinant DNA molecules that are refractive to cloning in common host strains may be enhanced in strain CES200.

Approximately 6% of the DNA from man (35), *Drosophila* (36), and *Physarum* (37) is accounted for by inverted repeats. In contrast to the random or clustered distribution of these sequences in *Drosophila* and the human genome, these foldbacks are regularly spaced at an average distance of 7 kb in the *Physarum* DNA (14), and several cloned, though unstable, *Physarum* foldback sequences were found to be highly repeated within the genome (38, 39). By electron microscopic analysis Hardman *et al.* (14) observed hairpins and stem-loop segments in about 90% of the reannealed DNA molecules, and most hairpin and stem structures measured about 180 base pairs. Such a distribution of inverted repeats might explain the previous problems of cloning 15- to 20-kb fragments of *Physarum* DNA in *E. coli* hosts that are not mutated in the *recBC* and *sbcB* genes.

By restriction mapping and Southern analysis of one recombinant phage, λPpA10, which contains the actin locus *ardA*, we found that the breakpoints of the 0.36-kb deletion are in close proximity or even within the actin-related sequence. It has recently been shown that an 86-base-pair foldback structure occurs in the coding sequence of the only gene isolated and sequenced from a genomic library of *Physarum*, that encoding histone H4 (5). This raises the question whether that gene is a pseudogene, containing a transposable element, or whether the foldback sequence appears in the transcript and is excised or perhaps causes instability of cloned cDNA.

Use of the isolated actin gene-homologous clones and M13 subclones as probes may allow determination of the distribution of the proposed foldbacks throughout the genome and their nucleotide sequence and examination of their possible function in genome replication (40), transcription (41), and transposition (42).

Note Added in Proof. After this paper was communicated, we learned that 8.9% of a human genomic library in a bacteriophage λ vector can be propagated in *recBC⁻sbcB⁻* mutant hosts but not in standard Rec⁺ hosts (43).

We acknowledge many stimulating discussions with Dr. Charles "Ned" Holt, who died February 15, 1982. This study was initiated by Dr. Gerard Pierron in our laboratory. We thank Dr. Gregory Shipley for discussion and help in preparing this manuscript; Drs. Terry Thomas and James Johnson for discussion, bacteriophages, and bacterial host strains; and Dr. Garret Ihler for support. We are indebted to Drs. Claire Shurvinton and Franklin Stahl (University of Oregon) for sending us their *E. coli* strain CES200 before publication and to Dr. Barbara Bachmann (*E. coli* Genetic Stock Center, Yale University) for other bacterial strains. We thank Mrs. Jung Choi and Mr. Jim Hernandez for excellent technical assistance and Mrs. Sendy Wilson for typing the manuscript. This work was supported by Grant HU 141/13 from the German Research Council (to A.H.) and by Biomedical Research Support Grant S07RR07090-18, awarded by the National Institutes of Health, and by National Science Foundation Grant PCM-8411124 (to H.W.S.).

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