Cellular Localization of Leucine-Binding Protein from Escherichia coli

Abstract. *Rabbit antibody against leucine-binding protein isolated from* Escherichia coli *K-12 has been prepared. This antibody has been used in conjunction with enzyme-labeled antibody to allow an immunocytochemical localization of leucine-binding protein in the* Escherichia coli *cell. This protein appears to be present only in the cell envelope and not in the cytoplasm.*

The treatment of bacterial cells with ethylenediaminetetraacetate and osmotic shock causes the release of several hydrolytic enzymes (*1, 2*). In addition, specific proteins which have been implicated in the active transport of inorganic sulfate (*3*), sugars (*4, 5*), and amino acids (*6*) are released by the same treatment. The ready release of the hydrolytic enzymes and binding proteins has suggested their localization near the bacterial surface (*1, 3, 6, 7*). Whereas there is electron microscopic evidence for the surface localization of the hydrolytic enzymes, alkaline phosphatase, cyclic phosphodiesterase, and acid phosphatase (*8*), no comparable evidence is available for the cellular localization of any of the binding proteins. This report provides such evidence for the localization of leucine-binding protein in the cell envelope of *Escherichia coli*.

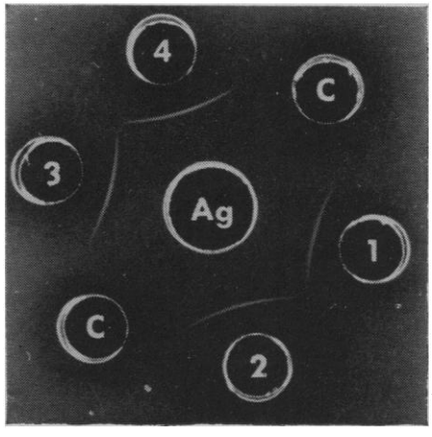

Fig. 1. Reaction between LBP and anti-LBP. Center well contains LBP (Ag); circumferential wells contain either control serums (C) or anti-LBP (1 to 4) from six different rabbits. Time of development was 36 hours.

Leucine-binding protein (LBP) was isolated and purified from early log phase *E. coli* K-12 cells grown on minimal medium C (*9*) with glucose as the carbon source (*6, 10*). The purified LBP was emulsified with Freund's adjuvant and injected into the foot pads of white New Zealand rabbits. The serums obtained 3 to 4 weeks after injection reacted with LBP in Ouchterlony double diffusion on agar to give single, sharp bands (Fig. 1); normal rabbit serums gave no reaction.

Rabbit antibody against LBP (anti-LBP) was used for ultrastructural localization of LBP by the enzyme-labeled antibody technique of Nakane and Pierce (*11*). This method has served to show that luteinizing hormone of rat pituitary is an intracellular tissue antigen and that basement membrane of mouse kidney is an extracellular tissue antigen (*11, 12*). Late log phase *E. coli* cells were fixed in cold acetone, washed in phosphate-buffered saline, and treated with rabbit anti-LBP. The cells were washed and allowed to react with sheep anti-rabbit gamma globulin which had been conjugated to horseradish peroxidase. After washing, the cells were stained cytochemically for peroxidase (*13*) which resulted in the deposition of reaction products at the antigenic sites. The cells were osmicated, embedded in Epon, sectioned, and observed in the electron microscope. This enzymatic method is particularly sensitive because of the amplifying effect of the peroxidase activity.

The cells were fixed in cold acetone, since either formaldehyde or glutaraldehyde fixation resulted in high background staining. Morphological abnormalities shown in Fig. 2 probably were caused by the acetone fixation.

As shown in Fig. 2, the cells treated with normal rabbit serums indicate no localization of LBP. In contrast, those cells treated with anti-LBP show the localization of LBP in the cell envelope; there is no indication of LBP in the cytoplasm. We could not determine whether the LBP was localized in the membrane, the cell wall, or in a region between the cell wall and the cytoplasmic membrane. These results are especially pertinent with regard to the implied role of LBP (*6*). The placement of LBP in the cell envelope supports the interpretation that LBP is a component of the transport system for the branched-chain amino acids in *E. coli*.

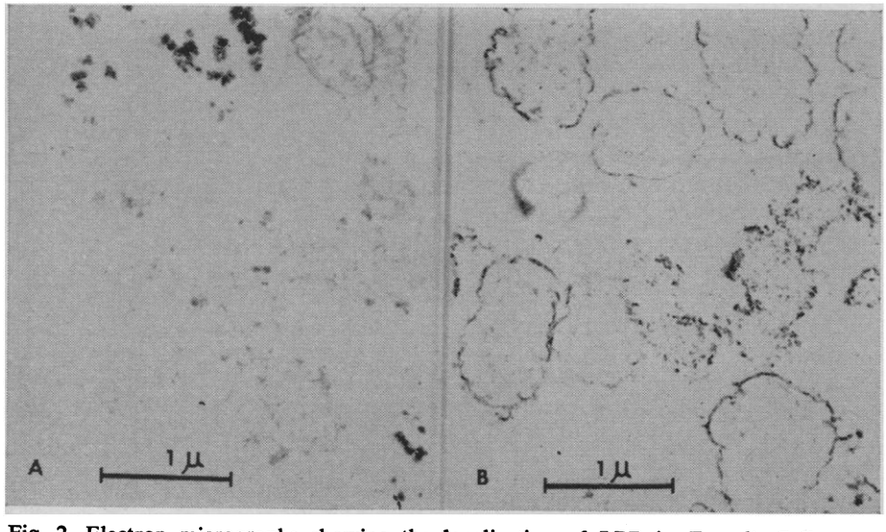

Fig. 2. Electron micrographs showing the localization of LBP in *E. coli*. Cells were treated either with control serums (A) or with anti-LBP (B) and then treated as described in the text.

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Multiple Chromosome Triplication in Liatris

Abstract. *A plant with four and one with six extra A chromosomes were discovered in a hybrid swarm involving* Liatris aspera *and* Liatris spicata. *In each plant, the extra chromosomes formed trivalents with chromosomes of the normal complement. This pairing behavior indicates that of the ten chromosomes in the complement the plants are triplicate for four and six chromosomes, respectively.*

Trisomics are known from numerous plant genera. They occur in natural populations and have been produced experimentally. The number of extra and different chromosomes which a diploid genome can tolerate rarely exceeds two (*1*). I now report the occurrence of four-chromosome and six-chromosome trisomics in *Liatris* (Compositae). To my knowledge, this degree of chromosome imbalance at the diploid level has never been described.

Two hybrids with several extra chromosomes have been discovered in a complex population involving *Liatris aspera* and *L. spicata* located near Zion, Lake County, Illinois. Whereas the two species and their hybrids usually have 20 chromosomes (*2, 3*), one hybrid possessed 26 chromosomes and the other 24 chromosomes. The extra chromosomes were the same size as those of the normal complement, and they displayed similar chromatic features and coiling cycles. The extra chromosomes were synapsed intimately with the normal complement at pachytene and remained that way through diplotene and usually through diakinesis and metaphase I. They may be regarded as type A chromosomes. Surprisingly, the external morphology of the two hybrids was not rendered distinctive because of the extra chromosomes.

The pairing relationships of chromosomes at diakinesis in the plants with 26 chromosomes are presented in Table 1. The chromosomes of this plant were arranged in 25 different associations based upon various combinations of univalents (I), bivalents (II), trivalents (III), and quadrivalents (IV). Figure 1 illustrates 6II + 4III + 2I. The most frequent deviation from normal pairing is the trivalent which occurs in 99 percent of the pollen mother cells (PMC's) examined. In fact, trivalents were more frequent than bivalents. The trivalents formed several different figures, but rings of three were notably absent. The number of trivalents varied from one to six with a mean of 4.6 per PMC. The frequent occurrence of 4II + 6III and 3II + 6III + 2I strongly suggests that six different chromosomes were present in triplicate. The rare formation of quadrivalents is informative in this regard. Should two of the extra chromosomes have been of the same type, quadrivalents should be common. This plant does produce quadrivalents, but only at a rate similar to that of chromosomally balanced interspecific hybrids (*2, 3*). Chromosome-pairing relationships indicate that the plant in question is a sixfold trisomic.

The morphology of the ten chromosomes in the set is similar at diakinesis so that identification of all of the extra chromosomes was not possible. The SAT chromosome (satellite-bearing), which is recognizable because of its association with the nucleolus, was present in triplicate.

The absence of rings of three and chains of five chromosomes suggests that each trisomic element is of the primary type (*1*). However, in view of the hybridity of the plant and the small reciprocal translocations which differentiate the parental complements (*2, 3*), it is possible that one or perhaps two of the trisomic elements is tertiary. Figures of five may have dissociated prior to diakinesis because of the small size of the translocated segments.

The chromosome-pairing behavior of the 24-chromosome hybrid is presented in Table 2. The chromosomes were arranged in 14 different associations. As in the other aneuploid, trivalents were the most common deviation from normal pairing. All PMC's contained trivalents which averaged 3.2 per PMC. The frequent formation of 6II + 4III and 5II + 4III + 2I strongly suggested that four different chromosomes were present in triplicate. This interpretation was strengthened by the near absence of quadrivalents. It may be concluded that each of the extra chromosomes is different, and that this plant is a fourfold trisomic. Since rings of three and associations of five chromosomes were lacking, it is likely that each trisomic element is of the primary type.

The identity of three of the extra chromosomes is unknown. The fourth

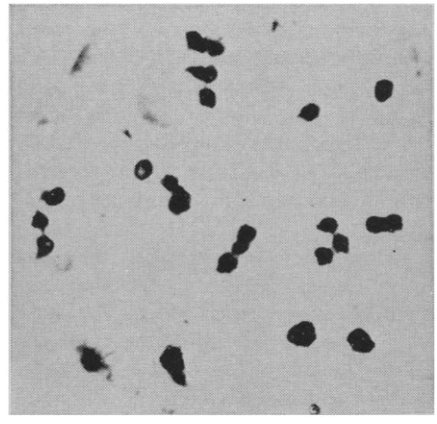

Fig. 1. The chromosomes of the sixfold trisomic; 6II + 4III + 2I (× 1350).

Table 1. Chromosome relationships at diakinesis in the plant with six trisomes.

Chromosome association	PMC (No.)	PMC (%)
10II + 6I	1	.5
9II + 1III + 5I	1	.5
8II + 1III + 1IV + 3I	1	.5
8II + 2III + 4I	2	1.0
7II + 3III + 3I	19	9.9
7II + 2III + 6I	2	1.0
6II + 4III + 2I	27	14.0
6II + 3III + 5I	5	2.6
6II + 3III + 1IV + 1I	2	1.0
6II + 2III + 8I	1	.5
5II + 3III + 7I	1	.5
5II + 5III + 1I	48	25.0
5II + 4III + 1IV	1	.5
5II + 3III + 1IV + 3I	1	.5
5II + 4III + 4I	9	4.7
4II + 6III	34	17.7
4II + 5III + 3I	19	10.0
4II + 4III + 6I	2	1.0
4II + 4III + 1IV + 2I	1	.5
3II + 6III + 2I	10	5.0
3II + 5III + 5I	4	2.1
3II + 4III + 1IV + 4I	1	.5
2II + 6III + 4I	1	.5
2II + 7III + 1I	1	.5
1II + 6III + 6I	1	.5

Table 2. Chromosome relationships in the plant with four trisomes.

Chromosome association	PMC (No.)	PMC (%)
9II + 1III + 3I	12	8.0
8II + 2III + 2I	8	5.3
7II + 3III + 1I	45	29.8
7II + 2III + 4I	1	.7
6II + 4III	48	31.8
6II + 3III + 3I	13	8.6
6II + 2III + 4I	3	2.0
5II + 4III + 2I	16	10.6
5II + 3III + 5I	1	.7
5II + 1IV + 3III + 1I	1	.7
4II + 1IV + 4III	1	.7
4II + 4III + 4I	1	.7
4II + 1IV + 3III + 3I	1	.7
3II + 5III + 3I	1	.7