Translation of Drosophila melanogaster sequences in Escherichia coli
(recombinant DNA/plasmids/protein synthesis/minicells/gel electrophoresis)

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ABSTRACT Thirty-seven independently cloned segments of Drosophila melanogaster DNA (Dm segments) were individually tested for their ability to promote the synthesis of new polypeptides in Escherichia coli K-12. The cloning vector was the pSC101 plasmid and the test system consisted of E. coli K-12 minicells that contained the hybrid pDm plasmids. Each of four pDm plasmids produced a new polypeptide, and one, pDm107, was selected for detailed mapping of the sequences required for the translation of its 38,000-dalton polypeptide, the Dm107 protein. Mapping was accomplished by constructing (i) deletion derivatives of pDm107 and (ii) new plasmids consisting of fragments of the Dm107 segment inserted into other vectors, and then testing these hybrids for their ability to promote the synthesis of the Dm107 protein, or truncated versions of this protein, in minicells. The 1000 base pairs of sequences that are translated to yield the Dm107 protein were thereby mapped at the center of the 18,000-base pair Dm107 segment, which consists of nonrepetitive sequences located at the base of the right arm of chromosome 2. The four polypeptides produced by the four pDm plasmids require sequences of 4000 base pairs for their translation, and the total amount of DNA in the 37 cloned Dm segments that were tested is approximately 400,000 base pairs. Because no new polypeptides were detected with the remaining 37 pDm plasmids, the fraction of D. melanogaster sequences that can be efficiently translated in E. coli K-12 is estimated to be \(1 \times 10^{-3}\).

Hybrid DNA molecules consisting of Drosophila melanogaster DNA segments (Dm segments) inserted into bacterial plasmids have been constructed in vitro and cloned by propagation in Escherichia coli K-12 (1, 2). Primary interest in these cloned segments has centered on the unparalleled advantages they offer for the molecular analysis of gene structure, organization, and expression in D. melanogaster chromosomes (1–7). By contrast, only scant attention has been paid to the basic question of whether structural genes carried by the cloned Dm segments can be expressed in the bacterial host. It is this question that we address here.

In the first part of this paper we describe experiments in which 37 independently cloned Dm segments were screened for their ability to promote the synthesis of new polypeptides in E. coli K-12. These segments had been inserted into the tetracycline resistance plasmid pSC101, to form hybrids that are called pDm plasmids. The pDm clones were unselected because one of our purposes was to obtain an estimate of the fraction of D. melanogaster sequences that can be translated in E. coli. Only four new polypeptides were observed as a result of the insertion of a total of approximately 400,000 base pairs (400 kb) of D. melanogaster DNA among the 37 pDm plasmids. One of the four plasmids that produced a new polypeptide was examined in detail to identify the sequences required for its synthesis. The mapping of these sequences within the Dm segment constitutes the second part of this paper.

MATERIALS AND METHODS

E. coli K-12 strains HB101 (8) and the minicell-producing P678-54 (9) have been described. AR1062 is a restrictionless (HsdR−) derivative of P678-54 that we made by phage P1 transduction. The plasmids pSC101, pSC105 (10), and pDm101-107 (2) have been described. Plasmids pDm500 to pDm831 were formed by the insertion of randomly sheared D. melanogaster (Dm) segments into pSC101 at its EcoRI site according to the dAdT connector method (1). Transformations were carried out as described by Glover (11). All plasmids containing Dm segments were propagated under EK1 and P2 containment conditions, as defined by the “National Institutes of Health Recombinant DNA Research Guidelines” (Fed. Reg., July 7, 1976).

Restriction endonucleases EcoRI (12), BamHI (13), HindIII (14), and Sat I (S. Goff and A. Rambach, unpublished isolation from Streptomyces stanford, ATCC 29415) were provided by S. Goff and prepared according to the indicated references. Lengths of DNA restriction fragments were obtained by agarose gel electrophoresis (5). Plasmid DNAs were isolated as described by Wensink et al. (1), except that the following simpler procedure was used for the analysis of the pDm107 derivatives (Fig. 2). Bacterial lysates were obtained as before (1), except that 0.05% Triton X-100 was used in place of 0.5% Brij 58. The lysates were heated to 65°C for 15 min, the white aggregates formed by the heating were removed by centrifugation (Sorvall SS 34 rotor, 10,000 rpm, 10 min), and 2 ml of the supernatant was made 0.25 M NaCl and 10% in polyethylene glycol (Carbowax 6000) by addition of 5 M NaCl and 40% polyethylene glycol. After overnight storage at 4°C, centrifugation (Sorvall SS 34 rotor, 5,000 rpm, 10 min) yielded a pellet that was suspended in 1 ml of 0.25 M NaCl/1.0 mM EDTA/10 mM Tris-HCl, pH 8.0, and then mixed with 2 volumes of ethanol. After overnight storage at –20°C, the mixture was centrifuged (Sorvall SS 12 rotor, 10,000 rpm, 10 min) and the pellet was suspended in 0.5 ml of 1.0 mM EDTA/10 mM Tris-HCl, pH 8.0 to yield a preparation that is sufficiently enriched in plasmid DNA to allow restriction analysis and transformation. This method has the advantage that it allows one to screen large numbers of clones.

Abbreviations: kb, 1000 bases or base pairs in single- or double-stranded nucleic acids; NaDODSO₄, sodium dodecyl sulfate; Dm, derived from Drosophila melanogaster; Tc⁸ and Tc⁵, tetracycline resistant and sensitive, respectively; Km³, kanamycin resistant.

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The bands produced by 33 of the 37 pDm plasmids can all be accounted for by the pSC101 bands or by the faint bands of the controls (e.g., pDm101, 103–106, 812–816; Fig. 1). The four remaining hybrids each exhibit a single, well-defined band that is not produced by pSC101 or the controls. Those obtained with pDm102 and 107 are indicated by the arrows in Fig. 1 and have apparent molecular weights of 29,000 and 38,000, respectively. The new bands formed by pDm820 and 829 (not shown) exhibit apparent molecular weights of 23,000 and 50,000. All but pDm820 were restested with a second preparation of minicells, and each reproduced the original result. In addition, minicells from two new clones of AR1062 [pDm107], obtained from a second purified preparation of pDm107 DNA, were examined and found to give the same autoradiographic pattern as that shown in Fig. 1. While five of the six pSC101 bands can generally be detected in the pDm autoradiographs, band III, which is the most variable in relative intensity, was not detected, or was barely detectable in 15 of the 33 pDm patterns that do not contain an extra band, and in one of the four that do.

Mapping the Sequence Required for the Synthesis of the Dm107 Protein. Among the four hybrids that generate a new band, the sequences in two (pDm102 and 107) had previously been characterized with respect to repetition frequency and location in the genome (2). Of these, pDm107 was chosen for further study because it promotes the synthesis of the larger polypeptide, which we chose to call the Dm107 protein for reasons that will become apparent. The Dm107 segment is 18 kb in length and consists of nonrepetitive sequences that are located at a single site in the right arm of chromosome 2 (region 41D).

To map the sequences required for the synthesis of the Dm107 protein, the cleavage sites for four restriction endonucleases were first located in the circular pDm107 DNA. The resulting map is shown at the top of Fig. 2. Deletion derivatives of pDm107 that lack one or more of the BarI, HindIII, or SalI restriction fragments were then constructed and cloned in HB101, as described in the legend to Fig. 2. Diagrams for six of these deletions are given below the pDm107 map. Each of
these deletion mutants was transferred to AR1062, and autoradiographs of the labeled polypeptides were prepared from the respective minicells in the standard manner. The right-hand column in Fig. 2 indicates the failure (−) or success (+) of each deletion in promoting the synthesis of the Dm107 protein.

All of the deletions that lack the H–J region of the Dm107 DNA are defective for this synthesis; hence, we conclude that part of all of the required sequences are located here. The H–J segment is 2.1 kb long, about twice the length required to code for a polypeptide of 38,000 daltons. pDm884 is not defective for Dm107 protein synthesis, and, because it lacks the adjacent D–G segment, none of the required sequences can be located here.

Additional mapping data were obtained from the synthetic capacities of hybrid plasmids consisting of the C–K Bam I fragment of Dm107 inserted into another plasmid vector, pSC105 (10). Fig. 3 shows that pSC105 consists of two EcoRI fragments: one is the pSC101 DNA, and the other contains a kan gene that confers kanamycin resistance (KmR) to its hosts. Cells carrying pSC105 are therefore TeR KmR. This is a useful vector because (i) it contains a single Bam I site, and (ii) insertion at this locus yields hybrids that produce TeR KmR transformants, evidently by inactivation of a tet gene. The desired hybrids were therefore obtained by screening TeR KmR transformants obtained from a ligated mixture of Bam I digests of pDm107 and pSC105 DNAs (Fig. 3 legend). Nine independently cloned hybrids consisting of the C–K Bam I fragment of Dm107 inserted into pSC105 were obtained in this manner. They divide into two classes: three have the orientation indicated for pkDm896/1 in Fig. 3, and six have the opposite orientation exhibited by pkDm896/2. The two classes were distinguished by electrophoresis of their HindIII fragments in 0.5% agarose gels, where the length of the δ+C fragment produced by pkDm896/1 is easily distinguished from that of the δ+K fragment of pkDm896/2 (Fig. 3).

pkDm896/1 and pkDm896/2 were transferred to AR1062, as was pSC105. Autoradiographs of the labeled polypeptides formed in the respective minicells are given in Fig. 3. Both of the pkDm plasmids reproducibly promote the synthesis of the Dm107 protein, indicating that all of the sequences required for this synthesis are contained within the C–K segment of Dm107. Because we have previously shown that sequences within the D–G segment are not required, those that are can be further localized to the C and H–K segments. This result is consistent with the previous conclusion that all or part of the required sequences are contained in the H–J segment.
following two experiments strongly suggest that the coding sequences for the Dm107 protein are confined to the H–J segment.

The ligation of the HindIII digestion products of pDm107 produces complex as well as simple deletions. pDm864 is such a complex deletion, and Fig. 4 shows that it differs from the simple pDm862 deletion by the insertion of the H–I segment, yielding a double deletion lacking the B–C and J segments. A comparison of the autoradiographs of the labeled polypeptides produced by pDm862 and 864 in AR1062 minicells indicates that the H–I insertion is responsible for the formation of two new polypeptide bands that migrate just in front of the pSC101 bands I and II—one strong band with an apparent molecular weight of 33,000 and a weaker band at 32,000.

The simplest explanation for these new polypeptides is that they resemble truncated Dm107 proteins whose translation is initiated normally in the H segment, proceeds through the I segment, and is prematurely arrested because the J segment is absent. If it is assumed that “false” translation of sequences lying outside of the H–I insertion contributes little to the 33,000-dalton polypeptide, then approximately 45 amino acids at the carboxyl end of the Dm107 protein should be coded by sequences in J; and, because only 135 of the approximately 345 codons required for 38,000-dalton Dm107 protein can be contained in J, the remaining 165 codons should be in H. Any contribution of false translation to the 33,000-dalton polypeptide will alter the calculation to increase the codons in J and decrease them in H. For example, the median expectation for terminator codons that are randomly distributed among the falsely translated sequences is that they will cause termination prior to the false translation of 15 codons, and application of this expectation will change the calculated number of codons in H and J to 150 and 60, respectively.

This explanation leads to a prediction that has been tested regarding the polypeptides expected to be produced by the A–D, E–H, and I–M EcoRI fragments of Dm107 when each is inserted into pSC101 (Fig. 5). Clearly, only the E–H plasmid should yield a new polypeptide, and its molecular weight should be approximately 18,000, the exact value depending upon the relative amounts of false translation for this and the pDm864 plasmid. Fig. 5 shows that each of four independently cloned hybrids containing the E–H fragment yields two bands migrating in advance of the pSC101 band VI—an intense band at 17,000 daltons and a weaker one at 18,000. It is curious that a pair of polypeptides differing in molecular weight by 1000 is produced by both this and the preceding presumptive truncation of the Dm107 protein. Perhaps the shorter member of each pair derives from the longer by loss of a common segment that is made sensitive to proteolytic cleavage by incomplete folding of the truncated polypeptides.

It should be emphasized that no other new polypeptide was produced by the hybrids containing the EcoRI fragments. The band that is seen between pSC101 bands I and II in all the autoradiographs of Fig. 4 does not result from the plasmids because it is also seen with the AR1062 control lacking plasmids, when, as was the case here, the second sucrose gradient centrifugation is omitted during purification of the minicells (see Fig. 1 legend). The band seen near the top of all the autoradiographs can be similarly discounted.

**DISCUSSION**

The linkage between the vector and Dm segments in the hybrid plasmids introduces some uncertainty in assigning the coding sequences for new polypeptides to the Dm segment. A new polypeptide may derive from translation of codons on both sides of a joint linking the two segments, or the joints may themselves create new initiation signals that lead to the translation of codons in either segment. We have eliminated these possibilities with respect to the synthesis of the Dm107 protein by excising the internal C–K fragment from the Dm107 segment, inserting this fragment at a different vector site in each of the two orientations, and then demonstrating that the two resulting hybrids retain the capacity to synthesize this protein (Fig. 3).

This result also restricts the *D. melanogaster* DNA sequences required for the synthesis of the Dm107 protein to the C–K fragment. These sequences have been further localized to the C and H–K regions by the observation that the capacity for this synthesis is not lost by deletion of the D–G fragment from pDm107 (Fig. 2). By contrast, deletion of the H–J region results in the loss of this capacity. Hence we know that the H–J region contains required sequences, whereas the C and K segments may, but do not necessarily, contain such sequences.

When the I and I–J segments are deleted from the right end of the critical H–J region, progressively shorter polypeptides...
of 33,000 and 17,000 daltons are synthesized in place of the 38,000-dalton Dm107 protein (Figs. 4 and 5). This shortening of the polypeptides is nicely correlated with the deletion lengths and, when taken in conjunction with the other mapping data, provides a strong argument that the Dm107 protein is translated from sequences within the H–J region—starting near the center of the 0.8-kb H segment, proceeding through the 0.4 kb of sequences in I, and finishing 0.1 to 0.3 kb within J.

Transcription of these coding sequences must then proceed from a promoter located to their left. That such a promoter resides within the C–K fragment is suggested by the equivalent amounts of the Dm107 protein generated by this fragment in both orientations (Fig. 3). The direction of transcription and the observation that deletion of the D–G region (Fig. 2) and of the B–C region (Fig. 4) does not prevent it, would further localize this promoter to the left half of the H segment, provided that the effective promoter in all of these hybrids is the same. Because we do not know that this is the case, such a placement is provisional.

Are these coding sequences transcribed and translated in D. melanogaster cells? We do not know. However, we think it unlikely that such a long row of approximately 94S sense codons would be maintained in the D. melanogaster genome unless it were expressed. We do not wish to imply that the primary transcript, the mRNA, or indeed the final protein product will be the same as those in E. coli. Rather we think that the coding sequence in the H–J region and the amino acid sequence in the Dm107 protein will overlap those in the RNAs and polypeptides produced by some D. melanogaster cells, and hence, that the kind of experiments contained in this paper represent a potential route for identifying certain genes contained in cloned eukaryotic DNAs.

Our data allow us to map another kind of sequence in Dm107—namely, that responsible for the diminution of the synthesis of the pSC101 band III polypeptide, a diminution that has been observed for many of the pDm hybrids, including pDm107 (Fig. 1). Fig. 5 shows that such sequences reside in the A–D region, and examination of other pDm107 deletions (e.g., pDm962; Fig. 4) indicates they can be further localized to the A segment. The fact that the sequence-specific effect of the Dm insertions on the synthesis of this polypeptide is quantitative rather than qualitative (Fig. 1) suggests that it results from a change in the concentration of transcripts containing its coding sequences, not from an interruption of their translation by an insertion among them. Sequences in the Dm segments could effect this change by altering the properties of a pSC101 promoter at or near the EcoRI site of insertion, or by providing signals either for the initiation of transcription of the band III gene in the wrong direction or for the termination of transcription that must proceed through the insert to transcribe this gene in the right direction.

Finally, we note that the four new polypeptides that we detected after testing 37 pDm hybrids that contain 400 kb of D. melanogaster DNA require approximately 3.8 kb of coding sequences for their translation. Evidently the fraction of D. melanogaster sequences that can be efficiently translated in E. coli K-12 is small. Even our estimate of \(1 \times 10^{-4}\) for this proportion may be too large if the coding sequences for the polypeptides generated by pDm102, 820, and 829 do not reside in the respective Dm segments. Others have recently observed new polypeptides in minicells containing pDm hybrids, but were unable to show that their codons reside in the Dm segment (18, 19); indeed, Meagher et al. (19) suggest that these codons derive, at least in part, from the vector DNA. While this estimate involves other uncertainties, including the fact that our scoring criteria will exclude certain polypeptides, we think it represents a useful first approximation, particularly because the other methods that have been used to detect the expression of eukaryotic DNAs in E. coli K-12 require extreme selective pressures (21, 22).

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