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ESCHERICHIA COLI dnaJ- AND dnaK-GENE PRODUCTS: SYNTHESIS IN MINICELLS AND MEMBRANE-AFFINITY

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SUMMARY: Escherichia coli dnaJ- and dnaK-gene products have been identified in a system of minicells infected with the appropriate transducing λ phages. The molecular weights of these polypeptides in dodecyl sulphate/acrylamide electrophoresis amounted to 39,000 and 77,000, respectively. Equilibrium sedimentation of minicell lysates in metrizamide density gradients has revealed that both these host proteins, indispensable for λDNA replication, are membrane-bound.

INTRODUCTION: The Escherichia coli dnaJ- and dnaK-gene products belong to a set of host proteins which have to interact functionally with one of the phage λ replication proteins, the P-gene product, for the proper λDNA replication in vivo to take place (1, 2, 3). The membrane-affinity, recently demonstrated, of the P protein (4) is compatible with the hypothesis that these interactions occur in an intimate association with the bacterial membrane. In this context it was reasonable to study the membrane affinity of the dnaJ and dnaK-gene products.

MATERIALS AND METHODS: The bacteriophages λdnaK and λdnaJ (λdnaJdnaKΔ14) were kindly given by Dr.H.Uchida (5); the phages λdnaJdnaK and λdnaJdnaKam3 - by Dr.C.P.Georgopoulos (6). Phage infection of minicells, labelling of proteins, lysis od minicells, metrizamide density gradient sedimentation and dodecyl sulphate/acrylamide electrophoresis were performed as described in (4).

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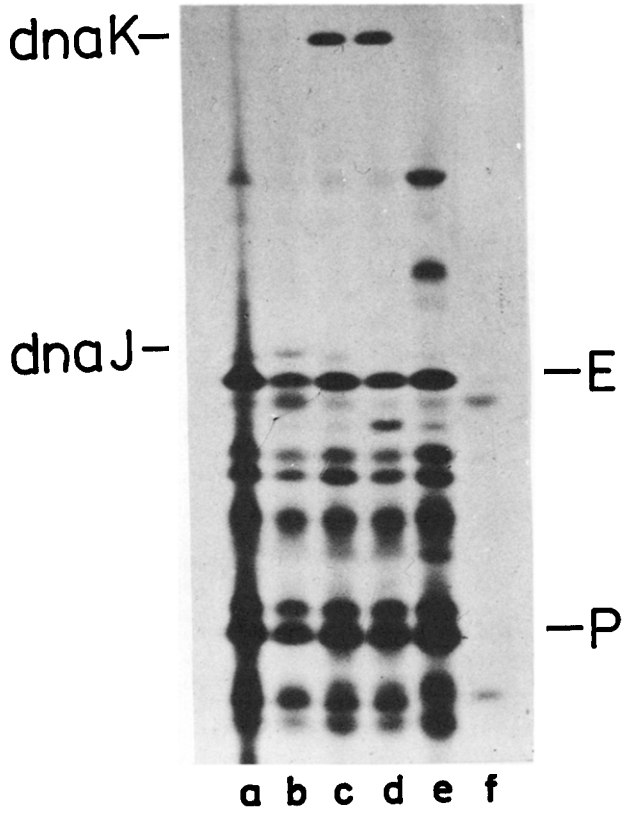

Fig.1. Electrophoretical pattern of polypeptides synthesized in nonpermissive (suO) E.coli minicells infected with transducing λ phages. The phages used for infection were: λdnaJdnaKam3 (a), λdnaJ (b), λdnaJdnaK (c), λdnaK (d) and λ⁺ (e). The last path (f) represents polypeptides synthesized in noninfected minicells. The ^{14}C-labelled polypeptides were separated in 10% polyacrylamide slab gel containing dodecyl sulphate and detected by fluorography. The bacterial gene products (dnaK and dnaJ), as well as some of the λ gene products (E, P) are marked.

RESULTS AND DISCUSSION: Performing the now classical identification of gene products in λ-infected minicells by the use of the appropriate amber or deletion mutants (Fig.1), we have noted that the apparent molecular weight of the dnaK-gene product is much smaller than the published value of 93,000 found in the system of λ-infected UV-irradiated bacteria (6). A detailed analysis (Fig.2) revealed that the apparent molecular weight of this polypeptide amounts to 77,000. Quite recently the identity of the dnaK-gene product

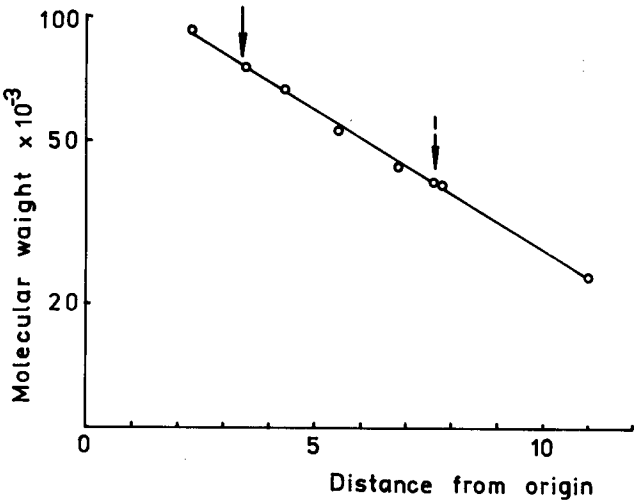

Fig.2. Log molecular weight versus mobility plot for the estimation of molecular weights of the E.coli dnaJ- and dnaK-gene products, following electrophoresis in 10% polyacrylamide gel containing dodecyl sulphate. Protein molecular weight standards were : phosphorylase b (94,000), transferrin (76,000), bovine serum albumin (67,000), glutamate dehydrogenase (53,000), ovalbumin (43,000), aldolase (38,900), λE-gene product (38,000), λP-gene product (23,000). The full and broken arrows indicate the mobilities of the dnaK- and dnaJ-gene products, respectively.

with B66.0 protein (M_r 75,000) of E.coli has been established (7). It seems, therefore, that the size of the dnaK-gene product synthesized in minicells is practically the same as in UV-irradiated bacteria. In our hands the dnaJ-gene product appeared as a M_r 39,000 polypeptide, close to the published value of 37,000 (8).

We have used the previously-elaborated gentle methods for minicell lysis and lysate fractionation : lysis by T4 lysozyme without detergents, and fractionation by equilibrium sedimentation in a metrizamide density gradient, both at low ionic strength (4). The λ-coded proteins synthesized in minicells, appear in two major fractions : as free proteins at the bottom of the centrifuge tube, and as membrane-bound proteins at the buoyant density of 1,18 - 1,22 g/cm^3 (4). Both E.coli proteins coded by the λ transducing phages

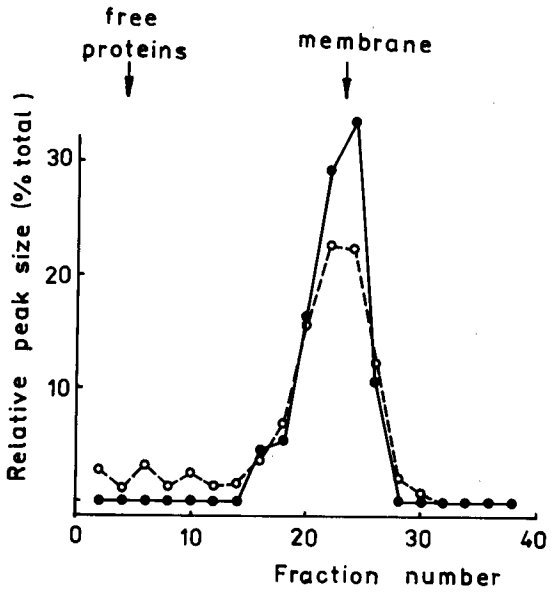

Fig.3. Profile of proteins, the bacterial dnaJ- and dnaK-gene products, synthesized in λdnaJdnaK-infected minicells, after metrizamide gradient sedimentation of the minicell lysate. The proteins synthesized in minicells were ^{14}C-labelled. Every second fraction from the metrizamide gradient was analyzed by dodecyl sulphate/acrylamide electrophoresis and the proteins were detected by fluorography. The relative peak sizes of the dnaJ - gene product (●) and of the dnaK-gene product (o) were determined by scanning the fluorogram and by integrating the areas under the peaks.

appeared predominantly (dnaK), or exclusively (dnaJ) in the membrane peak (Fig.3). In the presence of the ionic detergent, sarcosyl, both these proteins appeared in the fraction of free proteins (results not shown).

The above-demonstrated membrane-affinity of E.coli dnaJ- and dnaK-gene products, together with the membrane-affinity of the phage λ P-gene product (4) suggest that their interaction, inferred from genetic studies, may occur on the bacterial membrane, leading to an extremely effective initiation of λDNA replication in vivo. The recent success in establishing an apparently membrane-less in vitro system for λDNA replication (9) does not contradict this hypothesis; in spite of the use of highly concentrated bacte-

rial extracts, only 1 out of 10 molecules of λdv DNA was replicated at optimum conditions. The membrane-affinity of dnaJ-and dnaK-gene products may help in planning the strategy of preparative isolation of these proteins. With purified proteins and specific antisera it should be possible to check the role of dnaK- and dnaJ-gene products in the in vitro λDNA replication as well as in the in vitro replication of plasmids containing the E.coli chromosomal origin (10).

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