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속 보

A small RecA analog in *Streptococcus pneumoniae* that is not Induced during Competence for Genetic Transformation

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폐염균에서 작은 RecA 유사체의 검출 및 형질전환 때의 비유도성 확인

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ABSTRACT: Western blot analysis of lysates of *Streptococcus pneumoniae* revealed a single polypeptide species that cross-reacted with *E. coli* RecA antiserum. The apparent molecular weight of this putative RecA protein analog (RecAsp) was 24,000 smaller than any other known RecA analogue. The RecAsp protein was present at the same level in competent and non-competent cells.

KEY WORDS □ ***Streptococcus pneumoniae*, genetic transformation**

Competence for genetic transformation in *Streptococcus pneumoniae* (pneumococcus) is a specialized physiological state which appears in coordinated fashion in exponentially growing cultures, and lasts a few minutes (Morrison *et al.*, 1982). It is accompanied by synthesis of new proteins which are not made in noncompetent cells. During competence, donor DNA strands are transported into the cell and integrated at homologous sites in the chromosome. Competence specific proteins are thought to have significant roles in binding, uptake, and integration of donor DNA (Lacks and Greenberg, 1976; Morrison, 1977, 1978; Morrison and Baker, 1979; Morrison *et al.*, 1979, 1982; Morrison and Guild, 1973; Morrison and Mannarelli, 1979; Vijayakumar and Morrison, 1983, 1986), but biochemical characterization of these proteins is scanty, and the relationship between their activities and genetic exchange is not directly established.

In the well-studied *Escherichia coli* recombination system, the RecA protein (RecAec, molecular weight 37,842; Horii *et al.*, 1980; Sancar *et al.*, 1980) plays a central role in homologous genetic recombination (Radding, 1982) and in SOS repair (Walker, 1984). There is abundant evidence that proteins analogous to RecAec functions similarly in many other bacterial species (Better and Helinski, 1983; Eitner *et al.*, 1982; Finch *et al.*,1986; Goldberg and Melakanos, 1986; Hamood *et al.*, 1986; Hickman *et al.*, 1987; Keener *et al.*,Kokjohn and Miller, 1985, 1987; Lovett and Roberts, 1985; Miles *et al.*, 1986; Murphy *et al.*, 1987; Paul*et al.*, 1986; Pierre and Paoletti, 1983; West *et al.*,1983). Among the natur-

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ally competent organisms, the only one which is known to possess an SOS-like response (Campbell and Yasbin, 1984; Gasc *et al.*, 1980; Sicard, 1983) is *Bacillus subtilis*, a species that is distinguished from the others in that only a small percentage of the cells within a culture become competent (Nester and Stocker, 1963). *B. subtilis* is also the only naturally competent species in which a RecAec analog has been characterized. A gene capable of complementing *recA* mutations in *E. coli* was recently cloned from*Nisseria gonorrhoeae*, a naturally competent gram negative species, but its protein product has not been identified (Koomey and Falkow, 1987). Considering the importance of the functions attributed to the RecA protein and the ubiquity of RecAec analogs in other bacterial species, we expected the presence of a RecAec analog (RecAsp) in pneumococcus. The high efficiency of recombination in competent cells suggests that a RecAsp protein might be involved in competence, although the lack of an SOS response in this and other naturally competent organisms suggests that its control or function might be different from that of RecAec. In this study, we sought to identify a RecAec analog in pneumococcus, and to ask whether that protein is induced during competence.

The pneumococcus strain CP1200 was used for the preparation of lysates and competent cells. A single cross-reracting band was detected by Western blot analysis of lysates of competent of noncompetent cells (Fig. 1 and 2) performed with an anti- RecAec antiserum. Its apparent molecullar weight, 24,000 is only two thirds of that of the RecAec protein (molecular weight 37,842; Horii *et al.*, 1980; Sancar *et al.*, 1980). To test whether the 24 kilodalton (Kd) cross-reacting protein was a proteolytic product of a larger protein molecule, the concentration of the protease inhibitor, phenylmethylsulfonylfluoride (PMSF), was increased. No larger protein band was observed, even with 10 mM PMSF (Fig. 1, lanes 9 and 10). When *E. coli* RecA protein was added as an internal control before or after lysis of the pneumococcus cells, no proteolytic product of the RecAec protein was detected (Fig. 1, lanes 5-7).

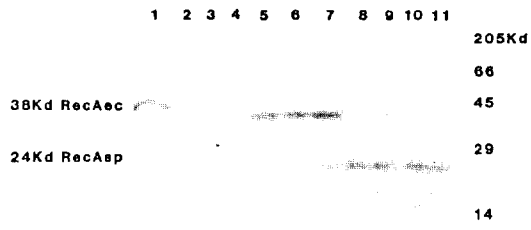

Fig. 1. *Identification of a 24Kd cross-reacting protein (RecAsp) in the anti-RecAec Western blot of lysates of S. pneumoniae.*
Culture conditions and lysate preparation were as described previously (Morrison and Baker, 1979) except that lysis buffer was supplemented with 1 mM PMSF, 1 ug/m*l* DNase I, and 1 ug/m*l* RNase. Lysates were boiled with 1% mercaptoethanol and 2% SDS, and then loaded on a 12% polyacrylamide gel for electrophoresis. Each lane contained protein from 2×10^8 cells. Western blot analysis employing anti-RecAec antibody, with peroxidase conjugated immunoglobulin G as a second antibody, was performed according to the procedure of the supplier of 4-chloro-1-naphthol, BRL (Gaithersburg, Maryland). Proteins were blotted to nitrocellulose, and analysed with antiserum to purified RecAec. Lane 1, pneumococcus culture supernatant + 1 ug RecAec; lanes 2 and 3, molecular weight markers; lane 4, 10 ng RecAec; lane 5, pneumococcus lysate + 1 ug RecAec (added before boiling); lane 6, pneumococcus lysate + 1 ug RecAec (added after lysis); lane 7, pneumococcus lysate + 1 ug RecAec (added before lysis); lane 8, pneumococcus lysate prepared by the standard procedure; lane 9, pneumococcus lysate prepared with 3 mM PMSF; lane 10, pneumococcus lysate prepared with 10 mM PMSF; lane 11, pneumococcus lysate prepared by the rapid SDS boiling method.

To explore further whether the 24Kd cross-reacting protein might be a proteolytic product of digestion by cell proteases, the pneumococcus cells were boiled in sodium dodecylsulfate (SDS) for 5 minutes immediately after resuspending in the lysis buffer at 0°C, to destroy any protease activity immediately on initiation of cell disruption. But this did not result in a higher molecular weight protein band on the Western blot (Fig. 1, lane 11). Finally, RecAec protein was mixed with a pneumococcus culture supernatant to determine whether the supernatant has a protease activity,

but no hydrolysed product of the RecAec protein was detected (Fig. 1, lane 1). In summary we found no indication either of degradation of the RecAec protein or of the existence of a larger form of the pneumococcal 24 Kd protein in any of these control experiments. We conclude that the 24 Kd cross-reacting protein is not a hydrolysed product of a larger protein arising as an artifact during lysis.

Essentially all of the known *recA* analog genes in other bacteria complement mutations in the *E. coli recA* gene both for DNA recombination and for DNA repair (Better and Helinski, 1983; deVos *et al.*, 1983; Eitner *et al.*, 1982; Goldberg and Melakanos, 1986; Hamood *et al.*, 1986; Hickman *et al.*, 1987; Keener*et al.*, 1984; Kokjohn and Miller,1985, 1987; Koomey and Falkow, 1987; Lovett and Roberts, 1985; Miles *et al.*, 1986; Murphy *et al.*,1987; Paul *et al.*, 1986; West *et al.*, 1983); also the *recAec* gene complements the defects of some of the *recA* analog genes (deVos *et al.*, 1983; Eitner *et al.*, 1982). All the RecA analog proteins from other bacteria tested to date cross-react with anti-RecAec antiserum (Hamood *et al.*, 1986; Keener *et al.*, 1984; Kokjohn and Miller, 1987; Lovett and Roberts, 1985; Murphy *et al.*, 1987; West *et al.*, 1983); we are aware of no report that a cloned *recA* analog gene product does not cross-react with antiserum raised against RecAec protein. Thus the *recA* gene product appears to be functionally and structurally conserved very broadly among the bacteria. This broad conservation leads us to conclude that the 24 Kd cross-reacting protein found in *S. pneumoniae* is likely to be an analog of the *E. coli* RecA protein (RecAsp), and to describe it here. This is the first RecA analog protein identified from a naturally competent organism other than *B. subtilis*. It is the smallest apparent RecA analog identified among the bacteria, as the apparent molecular weights of the RecA analogs from other bacteria reported to date range from 36,000 to 47,000 (Eitner *et al.*, 1982; Goldberg and Melakanos, 1986; Hamood *et al.*, 1986; Kokjohn and Miller, 1987; Koomey and Falkow, 1987; Murphy *et al.*, 1987; Paul *et al.*, 1986; Pierre and Paoletti, 1983; West *et al.*, 1983).

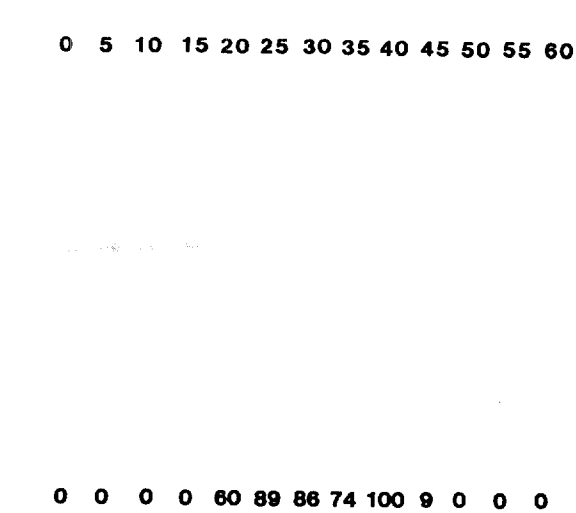

Fig. 2. *Lack of induction of the RecAsp during competence.*

S. pneumoniae cells were grown in the complete transformation medium, CTM (pH 7.0), to an O.D. of 0.1 and then chilled to 0 °C (Morrison *et al.*, 1983). The cells were frozen with 10% glycerol at -80 °C until needed. To examine a culture during development of competence, cells were thawed at 0 °C, and resuspended in same volume of CTM (pH 7.6) containing 0.5% competence factor. 10 m*l* samples were taken at 5 minute intervals during incubation at 37 °C. Lysate preparation, polyacrylamide gel electrophoresis, and Western blotting were performed as in Fig. 1. Induction of competence was measured by assaying DNA degradation, DNA uptake, and transformants (Morrison *et al.*, 1983) after 5 minute exposure to DNA. The time at which each sample was harvested is shown above each lane. The level of competence observed is indicated below each sample lane as the percent of maximum yield of transformants.

The molecular weight of the RecAsp protein is quite different from that of any of the reported pneumococcal competence specific proteins (Vijayakumar and Morrison, 1986) suggesting that RecAsp is not one of the more prominent competence specific proteins. To ask directly whether RecAsp is induced during competence, a competent culture was prepared as described previously (Morrison *et al.*, 1983). Competence was measured in successive samples taken before, during, and after competence induction. When lysates of these samples were examined by Western blotting, the amount of RecAsp was observed to be constant

(Fig. 2). No prominent protein band at the molecular weight of 24,000 was observed in a duplicate gel stained with Coomassie Brilliant Blue, suggesting that RecAsp is not a major cell protein.

In pneumococcus, competent cells are capable of a massive amount of homologous recombination. Therefore, RecAsp was a candidate to be one of the competence specific proteins and to be involved in recombination during competence. In *B. subtilis* , both the RecA analog protein (the *recE* gene product; deVos and Venema, 1982) and an SOS-like response (Love *et al.,* 1985) are induced during competence. Yasbin proposed that organisms more highly competent than *B. subtilis* should not utilize an "SOS-like" system in the regulation of competence development (Yasbin, 1985), because activation of the SOS systems in the majority of cells during competence might raise the mutation frequency in these organisms to intolerable levels (Yasbin, 1985). This suggests that the RecA analog protein in highly competent bacteria may provide functions needed in recombination yet not induce an SOS-like response. As a controlling point in the SOS induction pathway in other bacteria, disruption of the protease activity of the RecA protein would be an effective way to block the SOS response. The unusually small size of RecAsp may reflect retention of a recombinase fuctions, and loss of a protease function for induction of SOS-like response.

적 요

폐염균 용해액을 대장균 RecA 항혈청과 반응시켜 Western blot을 하였을때 하나의 polypeptide band가 관찰되었다. 이 RecA 유사단백질의 분자량은 24,000으로서 지금까지 알려진 다른 RecA 유사체보다 분자량이 가장 작았다. 이 RecA 유사단백질은 형질전환능이 나타날 때와 나타나지 않을 때 모두 같은 수준으로 존재하였다.

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