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Genetics

Switches in macromolecular synthesis during induction of competence for transformation of *Streptococcus sanguis*

(genetic transformation/inducible DNA binding protein)

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ABSTRACT **The induction of synchronous development of competence for genetic transformation in *Streptococcus sanguis*, by either endogenous or exogenous competence factor (CF), is manifested in the transient synthesis of a new set of at least 10 polypeptides, ranging from 14,000 to 51,000 in molecular weight. Eight polypeptides (E14, E16, E24, E28, E32, E37, E44, E51) appear early, and two polypeptides (L34, L42) appear 5–10 min later. One of the newly synthesized early polypeptides, E16, is shown to be a component of the presynaptic complex containing single-stranded DNA that is produced *in vivo* upon uptake of native donor DNA. Concomitant with this induced synthesis of competence-specific polypeptides there is a net decrease in RNA and protein synthesis but no change in DNA synthesis; donor DNA-binding ability and transformability reach maxima during the phase of diminishing macromolecular synthesis. Subsequently, donor DNA-binding ability and transformability decay at disproportionate rates as cells return to the normal state of macromolecular synthesis within one generation. Coincident with the induction of competence, the synthesis of a new RNA transcript of high molecular weight appears to be induced which continues during the restricted phase of total cellular RNA synthesis.**

Genetic transformation in many species of bacteria is dependent on a transiently acquired physiological state, called competence, of the recipient cells. Competence confers upon cells the ability to bind native DNA from the environment. Binding culminates, after a series of events, in the integration and expression of the exogenous DNA (1–3). The competent state is elicited in some bacterial strains by the synthesis of a small protein (competence factor or CF) which triggers a synchronous response in cells to undergo competence (4, 5). CF can be collected and purified from the supernatant of a competent population and, when added exogenously, can induce competence in physiologically noncompetent cells of the same or closely related species (6). These inductions require protein synthesis (7).

In *Bacillus subtilis*, development of competence is restricted to a small, biosynthetically inactive fraction (<20%) of the cell population (8), whereas in *Streptococcus sanguis* (9) and *S. pneumoniae* (10) nearly the entire population becomes competent for a short time during exponential growth. Until recently, no significant changes in overall macromolecular synthesis (11) have been detected in the latter group of species. Although induction of new proteins during development of competence has been demonstrated (12), more recent evidence indicates that protein synthesis during the development of competence in *S. pneumoniae* is restricted to a new set of 11 principal species (13).

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MATERIALS AND METHODS

Bacterial Strains. The bacterial strains used and the methods for obtaining unlabeled and [^{3}H]thymidine-labeled donor DNA preparations have been described (3).

Transformation. Recipient cells (3) were pelleted in the cold, resuspended in prewarmed competence medium (14) at 10 times the original volume, and incubated at 37°C. At intervals, samples were withdrawn, chilled, pelleted, and washed in leucine-deficient synthetic tissue culture medium (15) in the cold; then the cells were resuspended and incubated for a final 10 min of growth in synthetic medium supplemented with leucine at 30 μg/ml. This shift permitted a high level of incorporation of radioactive precursors (see below) without any effect on the growth rate or development of competence.

Macromolecular Synthesis. Cell samples removed from competence medium at various time intervals, in volumes to contain 1–2 × 10^8 colony-forming units (CFU) were pelleted, washed with chilled synthetic medium, and labeled for 10 min at 37°C in 0.5 ml of synthetic medium supplemented with [^{3}H]leucine (40 μCi/ml; 1 Ci = 3.7 × 10^{10} becquerels), [^{3}H]-uridine (100 μCi/ml), or [^{3}H]thymidine (20 μCi/ml). A portion from each sample was deposited on a filter disc (glass microfiber, GF/A). Each filter disc was then immersed in 10 ml of ice-cold 10% trichloroacetic acid for 4 hr, washed with 150 ml of 5% trichloroacetic acid and then 50 ml of ethanol, dried, and assayed for radioactivity.

Competence. Cells in 0.5 ml of synthetic medium were incubated for 8 min and exposed to 1 μg of [^{3}H]thymidine-labeled *S. sanguis str-r43* (streptomycin-resistant) DNA (specific activity, 2.0–2.2 × 10^5 cpm/μg) for 1 min. DNA uptake was terminated by addition of DNase I (20 μg/ml) and incubation for an additional 1 min at 37°C. Samples were then kept on ice. To determine DNase I-resistant donor DNA uptake, a portion (0.4 ml) from each sample was deposited on a filter disc (GF/A), washed with 100 ml of ice-cold 0.15 M NaCl, dried, and assayed for radioactivity. The remaining portion was plated, after appropriate dilution, under nonselective conditions to estimate total CFU and transformants as described (16).

Protein and RNA Analysis. Cell samples from competence medium were pulse-labeled with [^{3}H]leucine (40–120 μCi/ml) in synthetic medium for 10 min at 37°C. After one washing with chilled lysin buffer (3) and then with spheroplast buffer [10 mM Tris·HCl, pH 8.1/1 mM EDTA/0.2 M NaCl/10 mM sodium azide/20% (wt/vol) sucrose], cells were finally resuspended in 20 μl of double-strength SB buffer. Homogeneous (electrophoretically pure protein) C phage lysin (0.1 μg in 10 μl) was then added to each sample and the mixtures were in-

Abbreviations: CF, competence factor; CFU, colony-forming units; E, early; L, late.

cubated at 0°C for 5 min and at 10°C for 5 min. The spheroplasts thus formed were lysed by addition of sarcosyl (0.6%) and an additional 5-min incubation at 10°C. All samples were then freeze-dried.

Freeze-dried samples were resuspended in sample buffer [65 mM Tris, pH 6.8/5%(vol/vol) 2-mercaptoethanol/2% (wt/vol) $NaDodSO_4$/10% (vol/vol) glycerol/0.02% bromophenol blue] and heated at 100°C for 2 min. The proteins were resolved by electrophoresis in a discontinuous buffer system of Laemmli (17) through stacking (4%) and resolving (11% or 12%) acrylamide slab gels (0.7 mm thick). Gels were stained with Coomassie brilliant blue, destained, and prepared for fluorography (18). Exposure to x-ray films (Kodak, X-Omat XR-5) was for 2–10 days at −80°C.

For RNA analysis, cells were pulse-labeled for 10 min at 37°C in synthetic medium supplemented with [^{3}H]uridine (100 μCi/ml), converted into spheroplasts, and lysed as described above except that lysis of the spheroplasts was achieved at 2°C for 10 min with an equal volume of a mixture of detergents (0.6% sarcosyl, 0.1% Na desoxycholate, and 0.2% Brij-58) and diethyl pyrocarbonate (final concentration, 0.1%). RNA was resolved by electrophoresis in agarose-methyl mercury gels (19) and visualized by ethidium bromide staining and also by fluorography.

Exogenous CF-Induced Development of Competence. Challis strain-specific CF was kindly provided by Jon M. Ranhand. Cells were grown in competence medium for 10 min at 37°C and divided into six equal portions (2.5×10^8 CFU each) which were then incubated for an additional 20 min at 37°C. During this period, either no CF or 20% (vol/vol) CF was added at various times. Samples were then pelleted in the cold, washed and resuspended in chilled synthetic medium, and split into two halves. One half was incubated and assayed for competence (see above). The other half was pulsed with [^{3}H]leucine for 10 min at 37°C and then prepared for $NaDodSO_4$/polyacrylamide gel electrophoresis.

Characterization of Protein in Presynaptic Complex. Cells in the early phase of competence development (55 min after transfer to competence medium) were chilled, pelleted in the cold, washed and resuspended in chilled synthetic medium, and split into two portions. One portion (0.5 ml) (2.5×10^8 CFU) was labeled with [^{14}C]thymidine (4 μCi/ml) for 8 min, exposed to [^{3}H]thymidine-labeled donor DNA (see above) for 1 min and to DNase I for 1 min at 37°C, and then left on ice. The other portion (1.0 ml) (5×10^8 CFU) was labeled with [^{3}H]leucine (60 μCi/ml) for 8 min and split into two equal parts; one part was exposed to unlabeled donor DNA for 1 min and to DNase I for 1 min; the second was exposed to DNase I for 1 min at 37°C but not to donor DNA. Both then were left on ice. Cells in each sample were then pelleted in the cold, washed, and lysed. The lysates were dialyzed overnight against 10 mM Tris·HCl, pH 8.1/0.2 M NaCl/1 mM EDTA at 0–2°C, sedimented in 5–20% neutral sucrose gradients, and analyzed as described (3).

RESULTS

Macromolecular Synthesis During Spontaneous Development of Competence. In all the experiments to be described below, the recipient cells attained their maximal donor DNA-binding ability and transformability at a cell density of 4–5 × 10^8 CFU/ml, approximately three generations (generation time = 32 min at 37°C) after transfer to competence medium. The spontaneous development of competence, induced by endogenous CF, was measured physically as well as genetically. DNA-binding ability and transformability reached maxima after about three generations of growth; however, transformability lagged behind DNA binding ability and subsequently decayed at a faster rate (Fig. 1*A*; see also Fig. 4*A*). Both the increase and decrease of competence occurred during exponential growth of the population, as measured by optical density of the culture and CFU. This increase and decrease was accompanied by a decrease and increase respectively, in both RNA and protein synthesis; DNA synthesis was unaffected (Fig. 1*B*).

The changes in synthesis of cellular polypeptides pulse-labeled with [^{3}H]leucine were recorded fluorographically (Fig. 2). The synthesis of the majority of cellular polypeptides declined significantly after about 65 min, reached a minimum at 90 min, and then increased rapidly by 110 min. The extent of the decline varied with different polypeptides, the synthesis of some appearing to be terminated (see arrows, Fig. 2). In

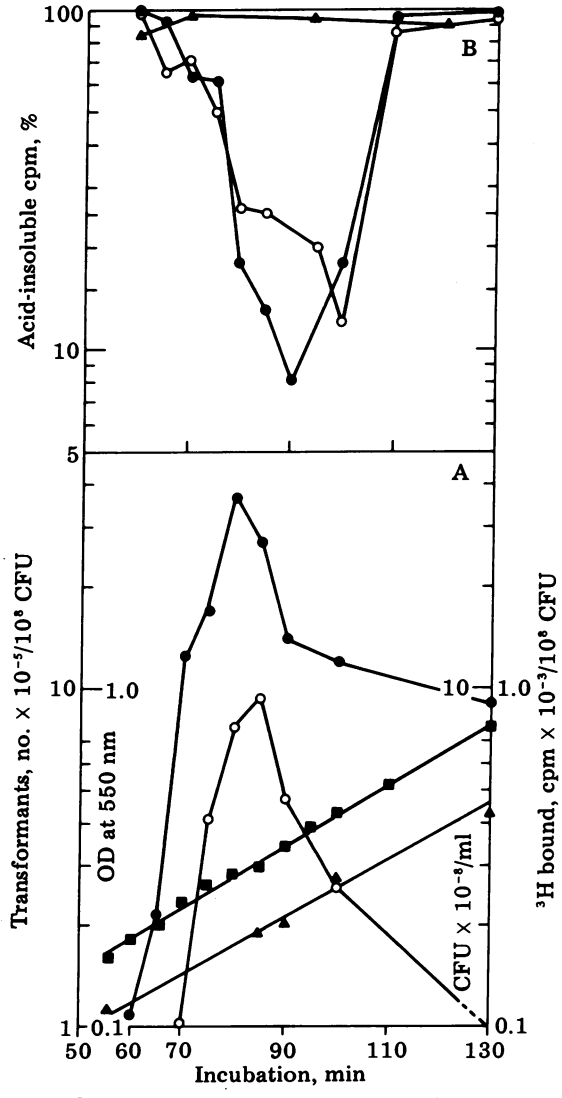

FIG. 1. Changes in macromolecular synthesis during growth and development of competence for genetic transformation. (*A*) DNA binding ability measured on DNase I-resistant, donor [^{3}H]thymidine-labeled DNA bound (●), transformability measured as *str-r43* transformants (○), and growth as increase in optical density at 550 nm (▲) and CFU/ml (■). (*B*) Macromolecular synthesis measured as rate of incorporation of [^{3}H]leucine (○), [^{3}H]uridine (●), and [^{3}H]thymidine (▲) into trichloroacetic acid precipitable material, expressed as percentage of [^{3}H]leucine and [^{3}H]uridine incorporated in 60-min samples and [^{3}H]thymidine incorporated in a 70-min sample. Elapsed time includes time at 37°C in competence medium plus 10 min in synthetic medium.

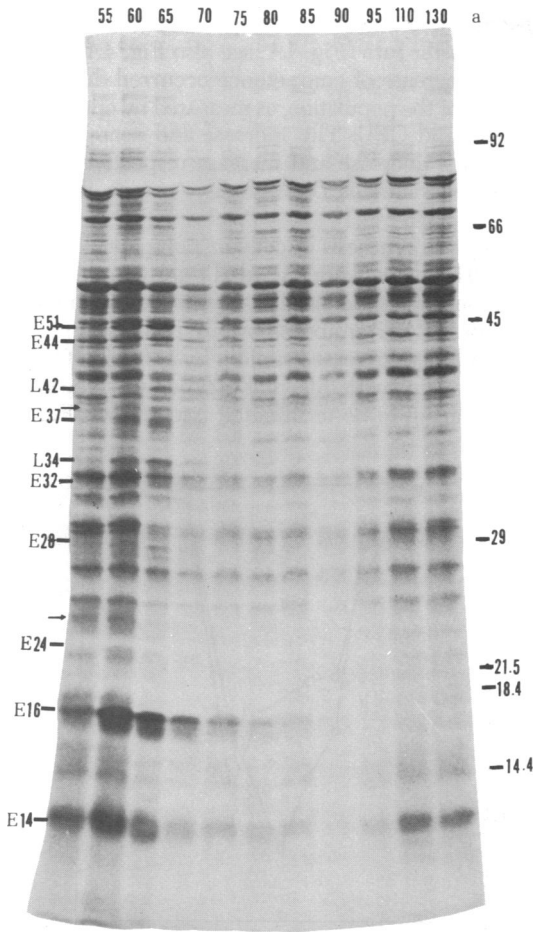

FIG. 2. Fluorograph of ^{3}H-labeled cellular polypeptides synthesized during endogenous CF-induced development of competence and analyzed in a NaDodSO$_4$/12% polyacrylamide gel. Lysates (2×10^8 CFU equivalent) of cells sampled at various time intervals are indicated by numbers (at top) corresponding to elapsed time (min) at 37°C in competence medium plus 10 min in synthetic medium. Competence-specific early and late polypeptides are denoted by letters E and L, respectively, and by numbers corresponding to their M_r ($\times 10^{-3}$) determined from the positions of reference proteins (lane a): phosphorylase A, 92,000; bovine serum albumin, 66,000; ovalbumin, 45,000; carbonic anhydrase, 29,000; soybean trypsin inhibitor, 21,500; β-lactoglobulin, 18,400; lysozyme, 14,400. Arrows (→) indicate the position of cellular polypeptides whose synthesis was markedly decreased during the development of competence (depicted more clearly in Figs. 3 and 4*B*).

addition, close examination of the fluorogram reveals that, upon the onset of the decline in protein synthesis, certain new polypeptides made their appearance (see, for example, bands E14, E16, E24, E28, E32, E37, E44, E51, L34, and L42).

The synthesis of this new group of polypeptides also is shown in the fluorogram obtained in a second similar experiment in which cell samples were examined at 45, 55, and 65 min after transfer to competence medium (Fig. 3). The late appearance of polypeptides L34 and L42 is clear. By the time the cells resumed their normal rate of protein synthesis, none of the competence-specific polypeptides were being synthesized (Fig. 2, lanes 110 and 130).

Induction by Exogenous CF. To determine whether the changes in macromolecular synthesis observed during the spontaneous development of competence were in fact triggered by CF, similar experiments were undertaken with cells stimulated by exposure to exogenous CF at a cell density at which spontaneous competence does not occur. Both DNA binding ability and transformability increased rapidly as a function of

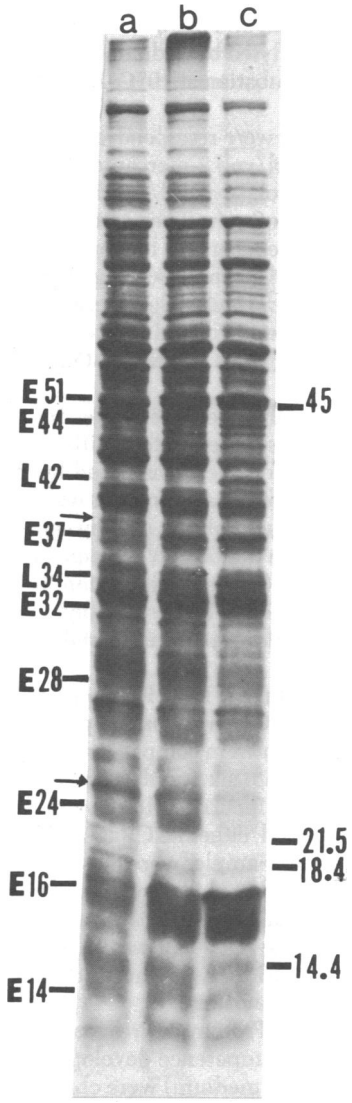

FIG. 3. Sequential induction of competence-specific early and late polypeptides. Details as in Fig. 2, except that [^{3}H]leucine-labeled lysates were analyzed on NaDodSO$_4$/11% polyacrylamide gel. Sampling times were 45 min (lane a), 55 min (lane b), and 65 min (lane c) at 37°C. Competence-specific early (E) and late (L) polypeptides, protein standards, and arrows (→) are as in Fig. 2.

exposure to CF, becoming maximal at 10 min for DNA binding ability and at 15 min for transformability (Fig. 4 *Left*). Control cells not treated with CF acquired these properties at a higher cell density (5×10^8 CFU) (see Fig. 1*A*). Fig. 4 *Right* shows the results of the fluorographic analysis of the polypeptides synthesized during the induction of competence by exogenous CF. The pattern of sequential changes is similar to that obtained during spontaneous induction of competence. Indeed, the pattern observed with exogenous CF usually was more sharply distinguishable than in the case of endogenous CF (spontaneous induction), presumably because of the higher degree of synchronous response achieved in the population.

Calculations of the rate of [^{3}H]leucine incorporation into acid-precipitable material revealed that protein synthesis declined to about 40% of the original rate within 10 min after the cells were in contact with CF. As seen in the spontaneous induction of competence, moreover, the synthesis of certain pre-induction cellular polypeptides (Fig. 4 *Right*, arrows) was more strongly diminished than others. The synthesis of competence-specific polypeptides showed a sharp decline after 20 min of contact with CF, and the decay in transformability was

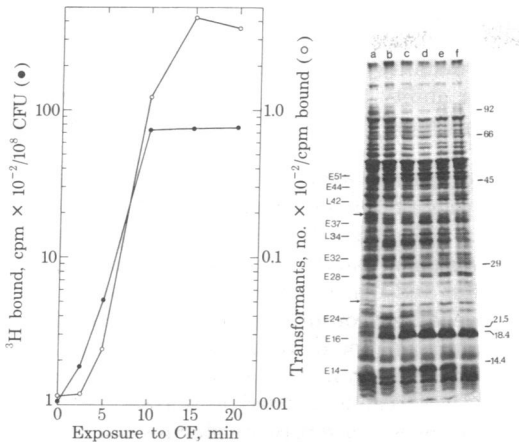

FIG. 4. Qualitative and quantitative changes in protein synthesis during exogenous CF-induced development of competence for genetic transformation. (*Left*) Development of competence. (*Right*) Fluorograph of NaDodSO$_4$/11% polyacrylamide gel: control (no CF) cell lysate (lane a) and lysates of cells exposed to exogenous CF for 2.5 min (lane b), 5 min (lane c), 10 min (lane d), 15 min (lane e), and 20 min (lane f) at 37°C. Competence-specific early (E) and late (L) peptides, protein standards, and arrows (→) are as in Fig. 2.

at a faster rate than the ability of cells to bind donor DNA (not shown), results similar to those obtained with endogenous development of competence (Figs. 1 and 2).

Characterization of Protein in Presynaptic Complex. Presynaptic complexes [single-stranded donor DNA–recipient protein complex formed after binding of native donor DNA to competent recipient cells (3, 20)] were prepared and analyzed as described in ***Materials and Methods***. The sedimentation profile of [^{3}H]thymidine-labeled presynaptic complexes is shown in Fig. 5 *Top*; all but a small fraction (donor DNA synapsed with recipient DNA) sediment at a slower rate compared to recipient DNA. Fractions from parallel gradients containing [^{3}H]leucine-labeled presynaptic complexes were analyzed by fluorography.

A polypeptide of M_r 16,000 was present in those gradient fractions containing [^{3}H]leucine-labeled presynaptic complexes (Fig. 5 *Center*); this polypeptide is similar in size to one (E16) induced after either endogenous or exogenous CF-stimulation of cells (Figs. 2, 3, and 4). Its position in the gradient is solely determined by its binding to, and hence sedimentation with, donor DNA because, in the analysis of [^{3}H]leucine-labeled competent recipients not exposed to donor DNA, none of the cellular polypeptides sedimented to a similar position in the gradient (Fig. 5 *Bottom*). The estimated M_r of 16,000 is similar to that of the M_r 15,500 polypeptide previously identified by *in vitro* ^{125}I-labeling of purified presynaptic complexes (20). Based on the densitometric tracings of Coomassie brilliant blue-stained gels relative to known protein standards (not shown), the average number of E16 polypeptides is of the order of 1–2 × 10^5 per cell.

Transcriptional Changes Associated with the Development of Competence. The distribution and relative abundance of various RNA species in cells undergoing spontaneous development of competence are shown in Fig. 6. Ethidium bromide staining revealed no readily perceptible changes in RNA patterns in cells before (lane a), during (lanes b–e), or after (lanes f and g) the development of competence. However, 10-min pulse-labeling of cells with [^{3}H]uridine followed by fluorography revealed two distinct features of RNA synthesis during the induction of the competent state: first, the overall rate of RNA synthesis decreased, reaching a minimum at about 95 min and returning to a normal rate at 130 min; second, a possible new transcript appeared (indicated by arrow in Fig. 6*B*) as the cells entered the competence phase (lane b), continued to be synthesized at a nearly constant rate (lanes c–e), and ceased to be synthesized (lane f) before the cells returned to a normal state of macromolecular synthesis (lane g).

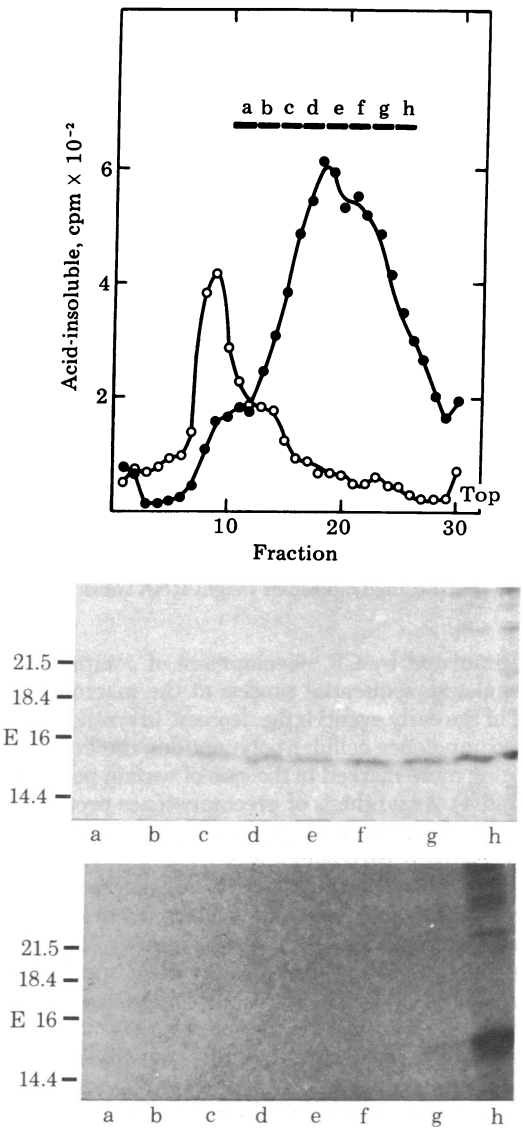

FIG. 5. Characterization of the polypeptide in presynaptic complex. (*Top*) Sedimentation profile of ^{3}H-thymidine-labeled donor (●) and [^{14}C]thymidine-labeled recipient (○) DNA in lysates of [^{14}C]-thymidine pulse-labeled recipients exposed to [^{3}H]thymidine-labeled donor DNA for 1 min. (*Center* and *Bottom*) Fluorographs of NaDodSO$_4$/11% polyacrylamide gels. Fractions, corresponding to those indicated by bars in *Top*, from parallel gradients in which lysates of [^{3}H]leucine pulse-labeled recipients exposed to unlabeled donor DNA (*Center*) or no DNA (*Bottom*) were analyzed. E16 refers to early competence-specific polypeptide (see Figs. 2, 3, and 4). Positions of reference proteins carbonic anhydrase (21,500), β-lactoglobulin (18,400), and lysozyme (14,400) are indicated.

DISCUSSION

Our analysis has revealed the considerable changes in macromolecular synthesis that accompany the acquisition by *S. sanguis* of competence to undergo genetic transformation. Like competence itself, however, these changes are transitory, the bacterial population reverting to its precompetence state of macromolecular synthesis 10–15 min after the competence phase.

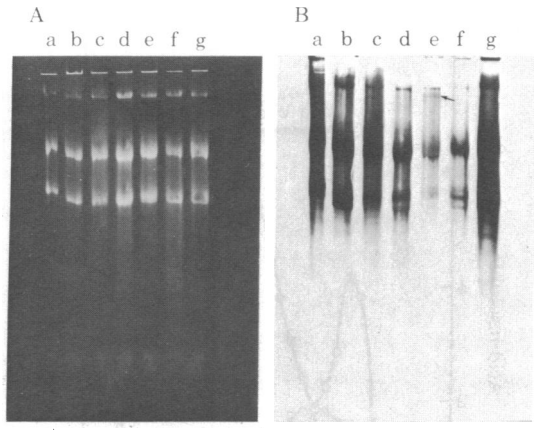

FIG. 6. Transcriptional changes associated with the development of competence: 2% agarose/methylmercury gel electrophoresis of [^{3}H]uridine-labeled cellular RNA. Lanes a–g correspond to sampling times, of 55, 65, 75, 85, 95, 110, and 130 min, respectively. (*A*) Ethidium bromide-stained gel. (*B*) Fluorograph (16-hr exposure at −80°C) of the same gel. Arrow in *B* indicates the position of newly synthesized, competence-specific, high molecular weight RNA transcript.

Once stimulated by CF, development of competence is a highly regulated, sequential process at the macromolecular level; one of the early events is the decrease in synthesis of most of the precompetence cellular polypeptides, the extent of the decline being more marked in the case of certain polypeptides (Figs. 2 and 4). As synthesis of precompetence proteins starts to decline, however, 10 new competence-specific polypeptides appear: 8 relatively early and 2 with a lag of 5–10 min (Figs. 2, 3, and 4). These changes in protein synthesis essentially may be attributed to a change in cellular RNA synthesis over the same time period (Fig. 6). Net RNA synthesis declines substantially, but the transcription of a new RNA species of high molecular weight appears to be selectively initiated and briefly maintained. This new species could account for the *de novo* synthesis of the competence-specific polypeptides, not only because transcription of the former and translation of the latter more or less coincide in time but also because the new transcript is large enough ($>2 \times 10^6$ daltons) to encode information for all of the competence-specific polypeptides.

Eventually, synthesis of the competence-specific polypeptides also declines as does the synthesis of other cellular proteins. When this general inhibition is released and protein synthesis reaches its original level, however, the competence-specific polypeptides are no longer made. RNA synthesis has also returned to the normal level and the competence-specific RNA species is no longer transcribed.

One of the early competence-specific polypeptides (E16) has been shown to bind specifically to donor DNA upon uptake (Fig. 5) and is similar in M_r to the M_r 15,500 polypeptide we have identified in presynaptic complexes formed *in vivo* and purified *in vitro* (20). It is clear that the competence-specific polypeptide E16 binds the undegraded strand of donor DNA (3). Presumably, it is involved in translocation of the donor DNA from the cell membrane to the chromosome (21, 22). How many of the other competence-specific polypeptides function in DNA binding and translocation to the chromosome is not clear, however. We are inclined to believe that at least some of the other competence-specific polypeptides are involved in the integration of donor DNA into the recipient chromosome via recombination reactions. This view stems from the observations that transformability always reaches its maximum 5–10 min after cellular ability to bind DNA is already maximal and subsequently decays at a much faster rate than DNA binding (Fig. 1*A*).

This kinetic uncoupling of DNA binding ability and transformability suggests that the integrative processes of transformation are as inducible as the DNA binding processes. Heretofore it was assumed that the induction of competence affected only the latter and that preexisting recombination or repair enzymes, needed for constitutive functions of the cell, could complete transformation by bound DNA.

A study of the macromolecular basis of competence of *S. pneumoniae* was recently reported by Morrison and Baker (13). Significant differences exist between their results and ours. First, a quantitative switch in protein synthesis, limited to a few specific polypeptides, occurs during the acquisition of competence in *S. pneumonia*, although a considerable decline in RNA and protein synthesis is characteristic of competence in *S. sanguis*. Second, a single polypeptide of M_r 19,500 constitutes the bulk of the synthesis of coordinately induced competence-specific polypeptides in *S. pneumoniae* but in *S. sanguis* the synthesis of sequentially induced early and late polypeptides is nearly uniform. Nevertheless, these inductions represent clear examples of cell differentiation in bacteria.

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