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12. Besides the defined major epitopes, there are minor T cell epitopes that represent less than 2 to 5% of primary responses. These minor epitopes specificities have been demonstrated in antiviral protection assays (*11*). Accordingly, H-2^d mice generate H-2–restricted cytotoxic T cells specific for LCMV-GP and H-2^k mice against LCMV-GP and NP, which can be boosted preferentially during secondary or tertiary responses in vitro. The effect of vaccination with recombinant viruses on a subsequent LCMV infection i.c. was studied also on days 10 and 60 to 70 after vaccination with slightly shifted kinetics, but with overall comparable results to the ones shown here for days 20 and 21.
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15. Evidence that CD8$^+$ CTL alone induce LCM has been shown by adoptive transfer of cloned T cells (*4*), by showing that class I–restricted effector T cells can cause LCM only if they recognize class I on infected choriomenigeal cells [P. C. Doherty, R. Ceredig, J. E. Allan, *Clin. Immunol. Immunopathol.* **47**, 19 (1988)], and by the in vivo depletion with antibodies to CD8 (*27*), indicating that T help is neither sufficient nor necessary to induce LCM. Also, CD8$^+$ T cell receptor transgenic mice that are specific for LCMV + D^b have increased CTL precursor frequencies that directly correlate with acceleration of LCM after infection i.c. (*9*).
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Altered Synaptic Plasticity in *Drosophila* Memory Mutants with a Defective Cyclic AMP Cascade

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Synaptic transmission was examined in *Drosophila* mutants deficient in memory function. These mutants, *dunce* and *rutabaga*, are defective in different steps of the cyclic adenosine 3′,5′-monophosphate (cAMP) cascade. In both *dunce* and *rutabaga* larvae, voltage-clamp analysis of neuromuscular transmission revealed impaired synaptic facilitation and post-tetanic potentiation as well as abnormal responses to direct application of dibutyryl cAMP. In addition, the calcium dependence of transmitter release was shifted in *dunce*. The results suggest that the cAMP cascade plays a role in synaptic facilitation and potentiation and indicate that synaptic plasticity is altered in *Drosophila* memory mutants.

SYNAPTIC PLASTICITY OCCURS THROUGHOUT the nervous system from neuromuscular junctions to central synapses (*1, 2*). It has been hypothesized that the cellular processes underlying learning and memory involve modulation of synaptic efficacy by second messenger systems (*3*). Genetic dissection in *Drosophila* provides a powerful tool for identifying and manipulating molecules that control synaptic efficacy (*4*). Among *Drosophila* mutants deficient in learning and memory (*5*), alleles of the *dunce (dnc)* and *rutabaga (rut)* loci have been subjected to extensive behavioral (*5, 6*) and biochemical (*7–10*) studies. Two *dnc* alleles, dnc^{M11} and dnc^{M14}, eliminate cAMP-specific phosphodiesterase II, leading to higher levels of cAMP-dependent phosphorylation in nervous tissues (*8, 10*). The rut^1 mutation abolishes Ca^{2+}-calmodulin (Ca^{2+}-CaM)–dependent activity of adenylate cyclase (*7*), disrupting Ca^{2+}-activated, cAMP-dependent phosphorylation of certain proteins (*10*). In spite of these behavioral and biochemical studies, it remains unknown if and how neural transmission is affected in these mutants. It is technically difficult to study synapses in the circuits for specific learning tasks in *Drosophila*. However, certain fundamental effects of an altered cAMP cascade on synaptic plasticity may be determined in synapses that are more accessible to physiological studies.

The two-microelectrode voltage-clamp method was used to study excitatory junctional currents (EJCs) at the *Drosophila* larval neuromuscular junction (*11*), which has been well characterized physiologically (*4, 12*) and anatomically (*13*). The muscle fiber was clamped at −80 mV (Fig. 1A) to prevent activation of voltage-gated ion channels (*14*). Therefore, transmitter release evoked at different external Ca^{2+} concentrations by nerve stimulation was accurately reflected by the EJC amplitude. The Ca^{2+} dependence of transmitter release in normal larvae thus determined (Fig. 1B) was consistent with that indicated by excitatory junctional potential (EJP) recordings (*12*).

The amplitude of nerve-evoked EJCs was enhanced in both dnc^{M11} and dnc^{M14} (Fig. 1). However, miniature EJCs (MEJCs) caused by spontaneous quantal release (*1, 15, 16*) remained unaltered in the mutants (Fig. 1A), suggesting a presynaptic defect. Instead of the fourth-power relation seen in normal larvae, the Ca^{2+} dependence of transmitter release in both *dnc* alleles (Fig. 1B), as reflected by either EJC amplitude or quantal content (*1*), is closer to a second- or third-power relation (power $n = 2.4$). The power (slope n in logarithmic plots) is usually interpreted to indicate the number of Ca^{2+} ions required in cooperative action to trigger transmitter release (*1, 16*). Thus, the change in *dnc* suggested a decline in Ca^{2+} cooperativity, which led to an increase in release probability (*16*). At a low Ca^{2+} concentration (0.1 mM), a large fraction of nerve stimuli led to failure in release in normal (0.59 ± 0.11, mean ± SD, four fibers) and rut^1 (0.55 ± 0.03, six fibers) larvae, but failure was rare in both *dnc* alleles (0.06 ± 0.06, three dnc^{M11} and three dnc^{M14} fibers, significantly different from normal, $P < 0.02$, and from rut^1, $P < 0.01$, by t test). In rut^1 larvae, EJCs were not significantly altered, displaying a Ca^{2+} dependence parallel to that of normal larvae (Fig. 1).

Facilitation was elicited with repetitive stimuli (2 to 20 Hz) at low external Ca^{2+} concentrations, resulting in a gradual increase in transmitter release. EJCs were recorded at 0.2 mM Ca^{2+} with 4-Hz stimulation (Fig. 2A). To characterize the frequency dependence of this process, we delivered 20 pulses at different frequencies, and averaged the last 10 EJCs from each fiber to determine their facilitated amplitude (Fig. 2B). In normal fibers, facilitation was

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not evident for stimulus frequencies below 2 Hz.

The initial EJC in *rut*^{1} was indistinguishable from that in normal larvae, but the facilitation in subsequent responses was much weaker (Fig. 2A), as reflected by the smaller EJCs at higher frequencies (Fig. 2B). In contrast, the initial response in *dnc*^{M11} was much greater than normal (Fig. 2A), but facilitation was suppressed at all frequencies (Fig. 2B). A similar defect was observed in *dnc*^{M14} and, to a lesser extent, in a mild allele *dnc*^{1}. An increase of external Ca^{2+} from 0.2 to 0.4 mM reduced the degree of facilitation in normal larvae but caused a clear depression of EJCs in *dnc*^{M11} (*17*) and, to a lesser extent, in *rut*^{1} (*18*). Depression was observed in normal larvae only when the Ca^{2+} concentration was further increased to 0.6 mM (*18*). Since elevated transmitter release might contribute to the lack of facilitation in *dnc* alleles at 0.2 mM Ca^{2+}, we examined facilitation in *dnc*^{M11} at 0.14 mM Ca^{2+}, a concentration at which synaptic release in *dnc*^{M11} was similar to that in normal larvae at 0.2 mM Ca^{2+} (Figs. 1B and 2). Although some facilitation was demonstrable in *dnc*^{M11} under this condition, the facilitation process was clearly abnormal because it was induced only with repetitive, high-frequency (> 8 Hz) stimulation (Fig. 2, A and B) and required a greater number of stimuli to attain a plateau level. The facilitatory effect and its decay after a single nerve stimulus was also abnormal in *dnc*^{M11} at 0.14 mM Ca^{2+}, as indicated by the twin-pulse experiment in Fig. 2, C and D. With this procedure, both *dnc*^{M11} (at 0.14 mM Ca^{2+}) and *rut*^{1} (at 0.2 mM Ca^{2+}) larvae showed reduction in the relative increment of the second EJCs at different intervals after the first EJC.

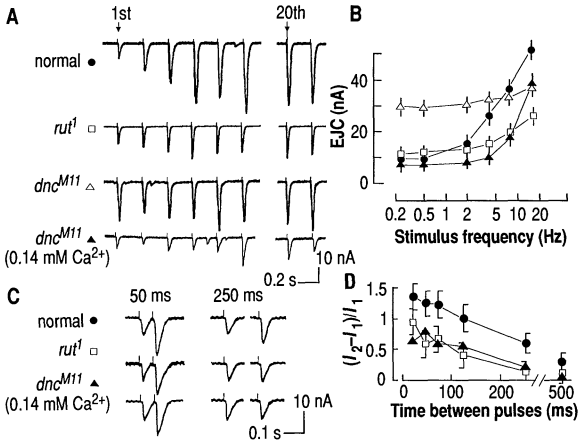

Fig. 2. Reduced facilitation in both *dnc*^{M11} and *rut*^{1}. (**A**) EJCs in response to 4-Hz stimulation recorded in normal, *rut*^{1}, and *dnc*^{M11} larvae at 0.2 mM Ca^{2+} and *dnc*^{M11} larvae at 0.14 mM Ca^{2+}. (**B**) Frequency dependence of EJCs in different genotypes at 0.2 or 0.14 mM Ca^{2+}. For each stimulus frequency, the last 10 EJCs in a train of 20 stimuli were collected from each of the five fibers in different larvae to obtain mean ± SEM. (**C**) EJCs in response to twin pulses (with interpulse intervals of 50 and 250 ms) recorded at 0.2 mM Ca^{2+} in normal and *rut*^{1} and at 0.14 mM Ca^{2+} in *dnc*^{M11} larvae. (**D**) Facilitation $[(I_2 - I_1)/I_1]$ as determined by the twin-pulse paradigm, where I_1 and I_2 represent the amplitude of the EJCs in response to the first and second stimuli.

Post-tetanic potentiation (PTP) can be induced by high-frequency, tetanic stimulation at low external Ca^{2+} concentrations. The PTP in *Drosophila* larvae was markedly different from that reported in the frog, in *Aplysia*, and in the crayfish (*1, 19*), where post-tetanic enhancement of EJCs varies with the duration of tetanus and decays exponentially. In contrast, PTP in *Drosophila* larvae appeared in an all-or-none manner after 15 to 20 s of tetanic stimulation (5 Hz). The induction of PTP was indicated by an abrupt enhancement of EJCs in response to test stimuli (0.5 Hz). Potentiated EJCs maintained a stable amplitude for minutes, then abruptly fell to the pretetanic level (Fig. 3). A longer tetanus only prolonged PTP duration without further increasing in the EJC amplitude.

After 50 s of continuous 5-Hz stimulation, the majority of normal fibers (Fig. 3A, trace a) produced PTP lasting from 40 s to 4 min (mean = 158 s, 18 fibers). Within the first second of tetanus, facilitation rapidly reached its maximum, followed by a gradual depression of EJCs. Depression did not develop during intermittent tetanic stimulation, but PTP still occurred (Fig. 3A, trace b). Thus, depression and PTP induction appeared to be independent processes.

At 0.2 mM Ca^{2+} and with 5-Hz stimulation PTP was never observed in 15 *dnc*^{M11} (Fig. 3A, trace c), 3 *dnc*^{M14}, and 6 *dnc*^{1} fibers or in 15 *rut*^{1} (trace d) and 6 *rut*^{1}/*Df(1)KA9* (trace e) (*20*) fibers (representative data are shown). The pretetanic EJCs in *dnc* were much larger than normal, as though they were already potentiated. Since *dnc*^{M11} showed some degree of facilitation at a reduced level of transmitter release, we examined its PTP again at 0.14 mM Ca^{2+}. Under this condition, PTP occurred only after the frequency of tetanus stimuli was raised from 5 to 8 Hz (Fig. 3A, trace f). However, the resultant PTP was short-lived (seconds as compared to minutes in normal larvae).

Pretetanus synaptic release was close to normal in *rut*^{1} and *rut*^{1}/*Df(1)KA9*. The EJC amplitude slowly increased throughout the 50-s tetanus but dropped back to the pretetanic level immediately after tetanus. When the stimulus frequency was raised to 8 Hz, facilitation was more pronounced during tetanus, but PTP was not elicited in any of the six *rut*^{1} larvae tested (*18*).

Long-term (>20 min) incubation of normal fibers in 150 μM dibutyryl cAMP, a membrane-permeable analog of cAMP, could partially mimic the effect of *dnc* mutations, that is, enhancement of pretetanic EJCs (Fig. 4). The effect of dibutyryl cAMP

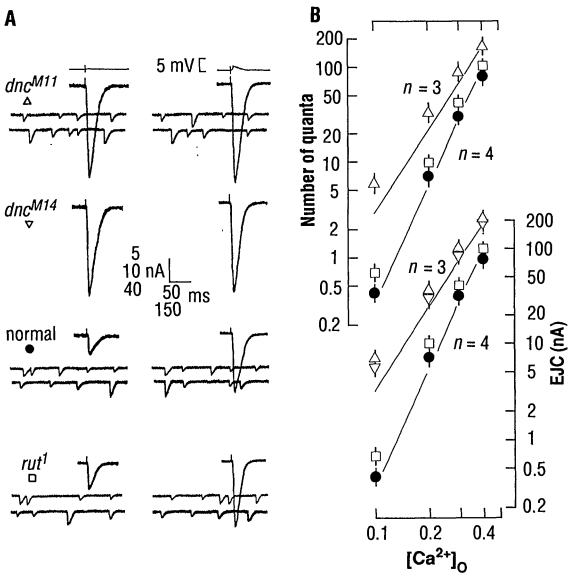

Fig. 1. Effects of *dnc* and *rut* mutations on EJCs and their Ca^{2+} dependence. (**A**) Spontaneous MEJCs and evoked (0.5 Hz) EJCs recorded at 0.2 (left four traces) and 0.4 (right four traces) mM Ca^{2+}. Among different genotypes and at different Ca^{2+} concentrations, MEJCs showed no statistically significant differences (mean ± SEM for samples shown at 0.2 and 0.4 mM Ca^{2+} were 1.1 ± 0.1 and 1.1 ± 0.1 nA for *dnc*^{M11} larvae; 1.1 ± 0.1 and 1.0 ± 0.1 nA for normal larvae; and 1.0 ± 0.1 and 1.0 ± 0.1 nA for *rut*^{1} larvae; n = 92 to 295). The voltage trace accompanying the *dnc*^{M11} EJCs represents the maximum extent of deviation from the holding potential (−80 mV) in these experiments. Current calibration: 5, 10, and 40 nA for MEJCs, EJCs at 0.2 mM Ca^{2+} and EJCs at 0.4 mM Ca^{2+}, respectively. Time calibration: 50 and 150 ms for EJCs and MEJCs, respectively. (**B**) EJC amplitude and quantal content (*1*) as a function of external Ca^{2+} concentration. Each data point represents the mean ± SEM of at least 40 responses (including failures) from each of four to five fibers in different larvae. The lines of slope n = 3 and n = 4 are drawn for comparison.

appeared to be activity-dependent because it could be speeded up and enhanced by high-frequency stimuli applied immediately after the dibutyryl cAMP treatments. Such enhanced EJCs did not show an appreciable decay for at least 30 min. Not until 15 min after wash did the EJCs gradually return to the level that prevailed before dibutyryl cAMP application (*18*).

Dibutyryl cAMP did not significantly affect EJCs in *rut*^{1} before tetanus. During the dibutyryl cAMP treatment, *rut*^{1} larvae partially regained PTP after tetanus, but the enhancement was lower than that reached in normal larvae and it decayed rapidly after tetanus (Fig. 4). At the Ca^{2+} concentrations of 0.2 mM (Fig. 4) and 0.14 mM (*18*), dibutyryl cAMP did not appear to modify EJCs in *dnc*^{M11} before, during, or after tetanus.

These results demonstrate that synaptic facilitation and potentiation are altered in both *dnc* and *rut* mutants, most likely because of the disrupted cAMP cascade. The lack of PTP and reduced facilitation in *rut*^{1} (Figs. 2 and 3) might be caused by failure in Ca^{2+}-CaM–dependent activation of cAMP production (*7*), and the higher cAMP level in *dnc* alleles (*5, 8*) might be responsible for the enhanced transmitter release (Fig. 1). Consistent with this explanation, dibutyryl cAMP increased EJC amplitude in normal larvae, partially restored PTP to normal in *rut*^{1}, but had no further effect on *dnc*^{M11} (Fig. 4). As shown in experiments on *dnc*^{M11} at 0.14 mM Ca^{2+}, facilitation and PTP are weakened not only by the failure of Ca^{2+}-dependent activity of adenylate cyclase in *rut*^{1} but also by the abnormally high levels of cAMP in *dnc* alleles (Figs. 2 and 3). The enhanced sensitivity to Ca^{2+} for transmitter release in *dnc* alleles further suppressed synaptic plasticity, as indicated by the observations at 0.2 mM Ca^{2+} (Figs. 1 to 3). Evidence that multineuronal innervation and proctolinergic terminals exist in some larval muscle fibers (*12, 13*) raises the possibility of heterosynaptic induction of PTP, but PTP was consistently induced in fibers with or without proctolinergic terminals (*18*). In addition, receptor-mediated adenylate cyclase activity is intact in *rut*^{1} mutants (*7*). Therefore, PTP is most likely induced homosynaptically.

It has been suggested that accumulation of residual Ca^{2+} from prior synaptic activity is responsible for facilitation and PTP in other species (*1, 19*). The defects in *rut*^{1} indicate that part of the residual Ca^{2+} effects may be mediated through Ca^{2+}-CaM–dependent activation of adenylate cyclase, which results in activity-dependent phosphorylation of proteins important to transmitter release, such as ion channels and other membrane-associated components (*3*).

Involvement of the cAMP cascade in synaptic modulation has been demonstrated in long-term facilitation at the crayfish neuromuscular junction (*21*) and in the sensitization process in *Aplysia* (*3*). The biochemical characteristics of cAMP binding are reported to be the same in different types of *Aplysia* neurons but different from those in muscle (*22*), and Ca^{2+}-CaM sensitivity may be common to all forms of neural adenylate cyclase (*23*). Therefore, memory and learning mechanisms may involve enzymes shared by different nerve cells (*22, 23*). Indeed, it has been reported that the sensory fatigue process of mechanoreceptors (*24*) and the number of nerve fibers in the mushroom body of the brain (*25*) are altered in both *dnc* and *rut* adults. Furthermore, growth-cone motility is reduced in cultured larval neurons of *dnc* and *rut* (*26*) and muscle K^+ currents in these mutants are affected (*17*). The phosphodiesterase and adenylate cyclase affected by these mutations may function in a variety of neurons and, therefore, synaptic plasticity in these neurons may display abnormalities similar to those observed at neuromuscular junctions.

Aging-induced changes in short-term facilitation and potentiation in the rat hippocampus correlate with impaired learning ability (*27*). Furthermore, electrically induced synaptic enhancement in the hippocampus blocks spatial learning ability in rats (*28*). The defects in synaptic facilitation and potentiation in *dnc* and *rut* mutants may thus lead to learning and memory deficiencies.

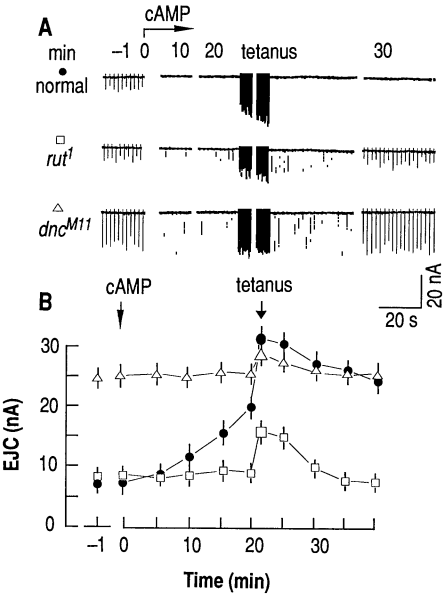

Fig. 4. (**A**) EJC traces before and after application of 150 μM dibutyryl cAMP in normal, *rut*^{1}, and *dnc*^{M11} larvae. To test the effect of dibutyryl cAMP, 20 stimuli at 0.5 Hz were delivered to the fibers every 5 min. After 50s of tetanus, 0.5-Hz stimuli were applied continuously. Data at −1, 0, 10, 20, and 30 min are shown. (**B**) Time course of dibutyryl cAMP effect and enhancing effect of tetanus. Each data point shows mean ± SEM determined from 15 to 20 consecutive responses in each of the three to four fibers in different larvae.

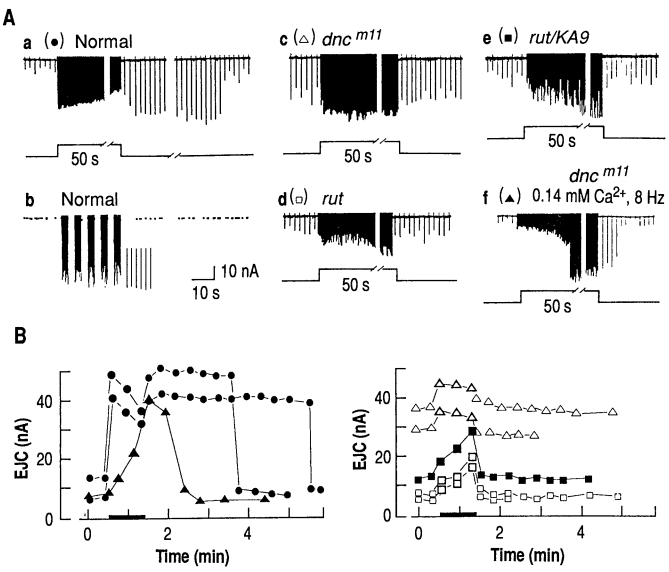

Fig. 3. Effects of *dnc* and *rut* mutations on PTP. (**A**) After 5-Hz tetanus stimuli, PTP was induced at 0.2 mM Ca^{2+} in normal (a) but not in *dnc*^{M11} (c), *rut*^{1} (d), or *rut*^{1}/*Df(1)KA9* (e) larvae (*20*). PTP was also induced at 0.2 mM Ca^{2+} with intermittent 5-Hz stimuli in normal larvae (b). At a lower Ca^{2+} concentration (0.14 mM) and increased stimulus frequency (8 Hz), *dnc*^{M11} larvae (f) were able to generate a short-lived PTP. (**B**) EJC amplitude before, during, and after tetanic stimulation (bar) of individual larvae were traced. Each data point is averaged from ten consecutive EJCs.

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Coordinate Regulation of β-Lactamase Induction and Peptidoglycan Composition by the *amp* Operon

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The *amp* operon, which is located on the *Escherichia coli* chromosome, modulates the induction of plasmid-borne β-lactamase genes by extracellular β-lactam antibiotics. This suggests that the gene products AmpD and AmpE may function in the transduction of external signals. β-Lactam antibiotics are analogs of cell wall components that can be released during cell wall morphogenesis of enterobacteria. The *amp* operon was studied to determine its importance in signal transduction during cell wall morphogenesis. The peptidoglycan compositions of *amp* mutants were determined by high-performance liquid chromotography and fast atom bombardment mass spectrometry. When a chromosomal or plasmid-borne copy of *ampD* was present, the amount of pentapeptide-containing muropeptides in the cell wall increased upon addition of the cell wall constituent diaminopimelic acid to the growth medium. These results suggest that β-lactamase induction and modulation of the composition of the cell wall share elements of a regulatory circuit that involves AmpD. *Escherichia coli* requires AmpD to respond to extracellular signaling amino acids, such as diaminopimelic acid, and this signal transduction system may regulate peptidoglycan composition in response to cell wall turnover products.

THE HETEROPOLYMERIC PEPTIDOglycan of *Escherichia coli* is composed of over 40 different building blocks (*1*). The overall composition of this exoskeleton requires coordination of the activities of ten synthetic enzymes, which attach the *N*-acetylglucosaminyl-*N*-acetylmuramyl pentapeptide precursors to the preexisting sacculus, and at least nine cell wall hydrolases, which manicure new cell wall during maturation and turnover. The control mechanisms governing this complex system are unknown but may involve coordinate regulation with the synthesis of the other cellular macromolecules (*2*). In addition, efficient communication must exist between the intracellular site of muropeptide precursor synthesis and the extracellular site of cell wall assembly. For example, *E. coli* has an efficient system for uptake and reuse of cell wall peptides released during turnover and maturation (*3*). Also, β-lactam antibiotics probably do not enter the cytoplasm, yet they induce intracellular events such as activation of cell wall hydrolases (*4*) and expression of β-lactamases (*5*). Because β-lactam antibiotics are structurally analogous to the D-alanyl-D-alanine component of the cell wall stem peptide, we reasoned that the pathway that leads to induction of β-lactamase production might share regulatory components with the pathway for cell wall metabolism. Therefore, we sought to obtain mutants with aberrant cell wall metabolism by selecting mutants that were altered in β-lactamase induction.

Constitutive overproduction of β-lactamase is associated with mutations in the *amp* operon (*5*). The operon contains two chromosomal loci, *ampD*, which encodes a cytoplasmic protein, and *ampE*, which encodes a membrane-associated protein (*6, 7*). To test the hypothesis that mutants altered in the regulation of β-lactamase induction would also show differences in cell wall metabolism, we analyzed two series of mutants in the *amp* operon (*8–10*) for the composition of their cell walls. We introduced plasmids that carried *ampD*, *ampE*, or both genes into these mutant cells to determine the ability of these loci to complement the chromosomal *ampD* and *ampE* defects.

Differences in peptidoglycan composition were apparent between the parents and the *amp* operon mutants (Fig. 1 and Table 1) (*11, 12*). Two peptidoglycan components with retention times of 56 and 82 min, which accounted for 27 ± 4.1% (± standard errors) of the total wall composition of the wild-type strain, were reduced to a total of <0.5 ± 0.4% in the absence of the *amp* operon. We determined, by fast atom bombardment mass spectrometry (FAB MS), that the masses of the peaks were consistent with those of monomeric and dimeric pentapeptides, respectively (that is, *N*-acetylglucosaminyl-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*meso*-diaminopimelyl-D-alanyl-D-alanine and this pentapeptide monomer linked to an identical unit that was missing the terminal D-alanine). The loss of these pentapeptide-containing species in the deletion mutant was accompanied by a proportionate increase in the monomeric and dimeric tetrapeptides (increased from 54 to 76%), such that the relative total amounts of monomeric and dimeric muropeptides in the wild-type and mutant strains remained

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