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REVIEW

Sex Pheromones and Plasmid Transfer in *Enterococcus faecalis*

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Plasmid-free *Enterococcus faecalis* excrete peptides (sex pheromones) which specifically induce a mating response in strains harboring certain conjugative plasmids. The response is characterized by the synthesis of a "fuzzy" surface material, visible by electron microscopy, which is believed to facilitate the aggregation of donors and recipients. Transconjugants which receive a specific plasmid shut down the production of endogenous pheromone; however, they continue to produce pheromones specific for donors harboring different classes of plasmids. In this review, we summarize what is known about the biochemistry and genetics of this phenomenon. Some emphasis is given to the hemolysin plasmid pAD1 and the regulation of its conjugal transfer.

Conjugative plasmids have been identified in numerous gram-positive bacteria, and many exhibit interspecies or intergeneric transfer (Clewell, 1981). One particular class of plasmids commonly found in *Enterococcus* (formerly *Streptococcus*) *faecalis* is unique in that a mating response is triggered by recipient-produced sex pheromones (Dunny *et al.*, 1978). The involvement of sex pheromones correlates well with the ability of plasmid DNA to transfer in broth matings; this contrasts with the case for many other gram-positive conjugative plasmids, including some in *E. faecalis*, which do not transfer in broth but exhibit broad host-range transfer in matings occurring on solid surfaces (filter matings). Pheromone-responding plasmids, examples of which are listed in Table 1, are the subject of this review.

E. faecalis, a Lancefield group D enterococcus, is a common inhabitant of the human gut. Strains frequently harbor plasmids determining drug resistance, hemolysins, and/or bacteriocins and may represent a reservoir of genetic information available to other bacteria in the intestine. A significant percentage of *E. faecalis* strains are hemolytic, a property frequently associated with a specific plasmid (see Table 1). Such strains are common among clinical isolates associated with human parenteral infections (Ike *et al.*, 1987), and there is evidence that the hemolysin contributes to virulence in a mouse model (Ike *et al.*, 1984). One such hemolysin plasmid is pAD1 (Table 1). Plasmids closely related to pAD1 have been identified in hemolytic isolates from widely separated geographical areas (LeBlanc *et al.*, 1983; Ike *et al.*, 1987; Colmar and Horaud, 1987); and it has been reported (Colmar and Horaud, 1987) that *E. faecalis* hemolysin plasmids form a single incompatibility group (IncHly). Most hemolytic isolates exhibit a similar pheromone-inducible mating response (Ike and Clewell, 1987). The pAD1 hemolysin plasmid will be referred to extensively in this review.

NATURE OF THE AGGREGATION EVENT

The pheromone response by donor cells requires about 30 to 40 min and is characterized by the synthesis of a proteinaceous substance that appears on the cell surface (Yagi *et al.*, 1983) and facilitates the formation of mating aggregates upon random collision with recipient bacteria. Induced surface material, collectively referred to as aggregation substance (AS),[1] appears as a fuzzy microfibrillar mate-

[1] Abbreviations used: AC, aggregation substance; BS, binding substance; LTA, lipoteichoic acid.

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TABLE 1

PLASMIDS KNOWN TO ENCODE A PHEROMONE RESPONSE

Plasmid	Size (kb)	Original host	Phenotype encoded[a]	Related pheromone	Related reference(s)
pAD1	60	DS16	Hly/Bac, uv	cAD1	Tomich *et al.*, 1979; Clewell *et al.*, 1982a; Dunny *et al.*, 1979
pPD1	56	39-5	Bac	cPD1	Yagi *et al.*, 1983; Dunny *et al.*, 1978
pAM373	36	RC73	?	cAM373	Clewell *et al.*, 1985
pCF10	54	SF-7	Tc^r(Tn*925*)	cCF10	Dunny *et al.*, 1981
pAMγ1	60	DS5	Hly/Bac, uv	cAD1	Clewell *et al.*, 1982b
pAMγ2	~60	DS5	Bac	cAMγ2	Clewell *et al.*, 1982b
pAMγ3	~60	DS5	?	cAMγ3	Clewell *et al.*, 1982b
pOB1	71	5952	Hly/Bac	cOB1	Oliver *et al.*, 1977; Clewell 1981; unpublished data
pJH2	59	JH1	Hly/Bac	cAD1	Jacob *et al.*, 1975; Clewell 1981; unpublished data
pBEM10	70	HH22	Pen^r, Gm^r, Km^r, Tm^r	cAD1	Murray *et al.*, 1988
pAM323	66	HH22	Em^r	cAM323	Murray *et al.*, 1988
pAM324	53	HH22	?	cAM324	Murray *et al.*, 1988

[a] Hly, hemolysin; Bac, bacteriocin; uv, ultraviolet light resistance; Tc^r, tetracycline resistance; Pen^r, penicillin resistance; Gm^r, gentamicin resistance; Km^r, kanamycin resistance; Tm^r, tobramycin resistance; Em^r, erythromycin resistance.

rial by immunoelectron microscopy (Yagi *et al.*, 1983; Galli *et al.*, 1989; Wanner *et al.*, 1989) and several immunologically unique proteins ranging in size from about 73,000 to 157,000 Da have been detected on induced cells (Kessler and Yagi, 1983; Tortorello and Dunny, 1985; Ehrenfeld *et al.*, 1986; Tortorello *et al.*, 1986). Aggregation of donors and recipients is believed to involve a "binding substance" (BS) which is present on the recipient surface and to which AS binds (Dunny *et al.*, 1979). Since donors alone will undergo an aggregation (clumping) response if exposed to the recipient pheromone, BS is believed to occur on the donor surface also. Aggregation requires phosphate ions and divalent cations (e.g., Mg^{2+}) (Yagi *et al.*, 1983). Lipoteichoic acid (LTA) may represent a component of BS because low concentrations (0.1 to 1.0 μg/ml) of added LTA are able to inhibit the aggregation (Ehrenfeld *et al.*, 1986). It is noteworthy in this regard that LTA corresponds to the Lancefield group D antigen on the *E. faecalis* surface (Wicken *et al.*, 1963).

Wirth and colleagues (Galli *et al.*, 1989; Wanner *et al.*, 1989) isolated a pAD1-related 78-kDa surface protein and raised polyclonal rabbit antibodies against it. Using direct immunogold labeling they were able to observe a dense layer of "hairs" on the surface of induced donor cells. Interestingly, the hairs were not evenly distributed. It appeared that the fibrils established only on the cell wall that was present at the moment induction began. That is, the new wall made during the 30–40 min required for full induction may not give rise to AS. Such a behavior suggests a convenient way for donor cells to dissociate from mating aggregates after plasmid transfer (i.e., cell division would quickly give rise to donors free of AS).

Suzuki and colleagues (personal communication) have also purified and characterized the major inducible pAD1-related surface protein as well as the major inducible protein of pPD1 (see Table 1). Antiserum raised against the latter specifically was able to prevent aggregation of induced pPD1-containing cells. Amino-terminal analyses of both proteins showed significant differences in amino acid sequence, a result consistent with their immunological dissimilarity.

MULTIPLE SEX PHEROMONES AND PHEROMONE INHIBITORS

A plasmid-free strain of *E. faecalis* (e.g., FA2-2 or OG1X) excretes multiple peptide pheromones (Dunny *et al.*, 1979; Clewell *et*

al., 1982b), specific for different conjugative plasmids. When a specific plasmid such as pAD1 is acquired by a recipient cell, it shuts down the production of the related endogenous pheromone cAD1. However, the cell continues to excrete unrelated pheromones specific for other plasmids. At least five different pheromones are known to be produced by a single cell, and it is believed that the production of many more are likely. Not surprisingly, *E. faecalis* strains may carry more than one pheromone-responding plasmid; strains DS5 (Clewell *et al.*, 1982b) and HH22 (Murray *et al.*, 1988) each carry three such plasmids (Table 1).

Plasmid-containing cells excrete a unique peptide that behaves as a competitive inhibitor (e.g., iAD1) of the pheromone to which the cells respond (Ike *et al.*, 1983). The inhibitor may serve to prevent induction by low levels of endogenous pheromone or by different pheromones with low levels of cross-reacting activity for the plasmid-specific pheromone receptor site. It may also act to prevent induction by pheromone levels too low to result in the generation of mating aggregates (i.e., recipients being too far away to encounter by random collision). There is evidence supporting the view that the inhibitor iAD1 is encoded by the plasmid pAD1. Specifically, when the plasmid was introduced into a strain which did not exhibit a cAD1 pheromone system (e.g., *E. faecium* 9790), iAD1 was excreted (Clewell *et al.*, 1987).

Several pheromones and some of the related inhibitor peptides have been purified and characterized by Suzuki and collaborators; their structures are shown in Table 2. All are hydrophobic octa- or heptapeptides. The pheromones have at least one hydroxyamino acid residue, which possibly could serve as a modification site for the shutdown (inactivation) of endogenous pheromone activity when the corresponding plasmid is present. A derivative of pAD1 with a 2.5-kb deletion has recently been found unable to shut down endogenous cAD1 production (Weaver and Clewell, 1988; see below); the specific shutdown mechanism remains to be determined. cAD1 and iAD1 are 50% homologous, with four of eight amino acid residues being identical (Table 2). cPD1 and iPD1 are only 25% homologous; however, iPD1 and iAD1 have three adjacent residues in common. It is noteworthy that the heptapeptide cCF10 has four adjacent residues in common with iAD1.

Synthetic sex pheromone and inhibitor peptides were found to have full activity and exhibited strong specificity for the related plasmid system. Deletion of one amino acid from the amino-terminus reduced cPD1 activity to 0.5%, and further deletions from this end resulted in essentially complete inactivation (Kitada *et al.*, 1985). Deletion of the carboxy-terminal residue of cPD1 diminished activity completely. In the case of cAD1, deletion of the amino-terminal residue resulted in no detectable activity, whereas deletion of the carboxy-terminal residue reduced activity to 0.02% (Kitada *et al.*, 1985). The full lengths of cPD1 and cAD1, therefore, are important to CIA activity.

Synthetic hybrid peptides corresponding to cPD1 on the amino-terminal half and cAD1

TABLE 2

STRUCTURES OF SEX PHEROMONES AND SOME RELATED INHIBITORS

Pheromone or inhibitor (M_r)	Peptide structure	Reference
cPD1 (912)	H-Phe-Leu-Val-Met-Phe-Leu-Ser-Gly-OH	Suzuki *et al.*, 1984
cAD1 (818)	H-Leu-Phe-Ser-Leu-Val-Leu-Ala-Gly-OH	Mori *et al.*, 1984
cAM373 (733)	H-Ala-Ile-Phe-Ile-Leu-Ala-Ser-OH	Mori *et al.*, 1986b
cCF10 (789)	H-Leu-Val-Thr-Leu-Val-Phe-Val-OH	Mori *et al.*, 1988
iPD1 (828)	H-Ala-Leu-Ile-Leu-Thr-Leu-Val-Ser-OH	Mori *et al.*, 1987
iAD1 (846)	H-Leu-Phe-Val-Val-Thr-Leu-Val-Gly-OH	Mori *et al.*, 1986a

on the carboxy-terminal half exhibited 10% the activity of cPD1 but had no cAD1 activity (Kitada *et al.*, 1985). The converse structure gave the opposite result, with 1% the activity of cAD1 and no cPD1 activity. Thus, the specificity of the peptide is determined by the amino-terminal sequences.

PLASMID TRANSFER

When donors are exposed to pheromone for 60 min and then mated with recipients for a short period of time (10 to 15 min), the frequency of transfer is generally 3 to 6 orders of magnitude greater than controls not previously induced. Transfer can occur readily between two homolgous donor strains if one of them is induced prior to the brief mating (Clewell and Brown, 1980). In such experiments where the two donors each had a distinguishable derivative of pAD1 (i.e., using the tetracycline-resistance transposon Tn*916* or the erythromycin resistance transposon Tn*917*) significant transfer occurred only in the direction from induced to uninduced cells. It was concluded from such experiments that in addition to inducing AS, genes necessary for DNA transfer were also induced. Had the induction of aggregation-related surface material been the only important pheromone-related response, one would have expected transfer in both directions between the two donors, regardless of which had been induced prior to the mating. Interestingly, when both donors were induced transfer occurred in both directions but at frequencies significantly reduced from the case where transfer was from an induced to an uninduced strain. Thus, entry (surface) exclusion also appears to be induced by pheromone. In the case of pCF10 Dunny *et al.* (1985) have reported evidence that a 130-kDa surface protein induced by pheromone (cCF10) is involved in surface exclusion. It was also shown that exclusion was specific in that entrance of pAD1 was not affected. A pheromone-induced surface exclusion would seem important in order to minimize exchange of plasmid DNA between homologous donors that are in close proximity to one another, along with recipients, in mating aggregates.

Consistent with the finding that genes other than those related to aggregation are also induced by pheromone is the observation that a bacterial strain harboring derivatives of both pPD1 and pAD1 is specific in its pheromone response (Ehrenfeld *et al.*, 1986). That is, when the cells were exposed to only cPD1 prior to a short mating, only the pPD1 derivative (marked with Tn*916*) transferred. On the other hand when the cells were induced with cAD1 only the pAD1 derivative (marked with Tn*917*) was found to transfer. These data implicate independent signal transduction pathways and are consistent with an absence of *trans*-complementation between unrelated, coresident, pheromone-responding plasmids.

INDUCIBLE CELL SURFACE PROTEINS

The pAD1 pheromone response involves the sythesis of at least four novel surface proteins that are readily detectable after 45 to 60 min (Ehrenfeld *et al.*, 1986). Using antiserum raised in rabbits against induced bacteria, proteins with M_r values of approximately 74,000, 130,000, 153,000, and 157,000 (referred to as AD74, AD130, AD153, and AD157, respectively) are observed. AD130, AD153, and AD157 strongly cross-reacted with similar bands that relate to the induction of pPD1 (PD130, PD153, and PD157). AD74 was the most prominant band produced and could be detected on gels stained with Coomassie blue. This band probably corresponds to a similar band (designated PD78) produced by pPD1-containing cells. AD74 and PD78 are immunologically distinct and represent the two major AS proteins that have been purified and characterized (see above). Similarly, Tortorello and Dunny (1985) detected several inducible surface antigens in the pCF10 system; these exhibited *Mr* values of 73,000 to 130,000. The smaller protein, designated SA73, was the most prevalent and may be analogous to AD74 and PD78.

GENETIC ANALYSIS OF pAD1

The transposon Tn*917* (Tomich *et al.*, 1980) has been useful in genetic analyses of

the pheromone-responding plasmids pAD1 and pCF10. In particular the construction by Youngman (1987) and colleagues of useful derivatives along with temperature-sensitive plasmid delivery vectors has been valuable (Weaver and Clewell, 1987). For both pAD1 and pCF10, inserts over large regions of the plasmid have been observed to affect mating. As shown in Fig. 1, insertions in pAD1 over a segment representing well over half of the plasmid exhibited significant effects on transferability (Ehrenfeld and Clewell, 1987). Insertions in regions D, E, F, G, and H greatly reduced or eliminated the ability of pAD1 to transfer in broth. Insertions in region H allowed cells to still undergo inducible aggregation, but plasmid transfer was not detectable in broth and was less than 0.7% of the wild-type in filter matings. Aggregation also was not affected by insertions in the adjacent G region; transfer, however, occurred at about 5% of wild-type levels in broth. All four of the inducible surface proteins could be detected for both G and H insertions. These two regions probably include determinants necessary for DNA transfer. Region F insertion derivatives did not undergo induced aggregation and/or were altered in the appearance of inducible surface proteins. In several cases both AD74 and AD157 were missing, but AD130 and AD153 appeared normal; in addition, a 140-kDa protein was readily evident. The latter may represent an increased level of an "AD140" barely detectable in the case of induced wild-type cells. One insert in region F exhibited only AD74 and a "new" 110-kDa protein. F-region mutants exhibited a reduced transfer (about 4% of wild-type) in broth, but wild-type transfer on filters, indicating that the ability to transfer DNA was not affected. The two inserts indicated by the arrows in Fig. 1 did not prevent induced aggregation. One insert (at 12.8 kb) resulted in undetectable levels of transfer in broth, but transfer occurred at wild-type levels in filter matings. This derivative produced only the AD153 surface protein. In another case (at 14.1 kb), transfer in broth occurred normally, despite the induction of only AD74 and AD153. Although it would appear that cells producing only AD153 are able to form aggregates in broth, the role (if any) of this protein in aggregation is more complex; region F insertions, which produced AD153 (or a similar-sized protein) along with AD130 and AD140, did not give rise to visible aggregates. It is not clear why certain variants not exhibiting AD74 (the presumed major AS component [see above]) still appear able to aggregate. Conceivably these cells have low, difficult-to-detect, levels of AD74 or have an unextractable, perhaps precursor, form of the protein. More work will be required to gain insight into the specific role played by the various surface proteins.

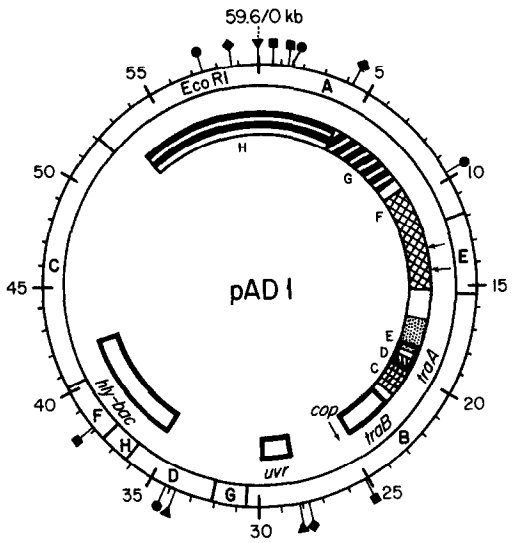

FIG. 1. Physical and functional map of pAD1. Regions important for transfer are indicated as shaded or open boxes on the interior of the map and labeled *traA, traB,* and C through H. Regions *hly-bac* and *uvr* represent genes for hemolysin-bacteriocin and uv resistance, respectively. *Eco*RI fragments are labeled within the outer circle. Other restriction sites are *Sal*I, ▼; *Bam*HI, ■; *Kpn*I, ♦; and *Pst*I, ●. This figure is identical to that shown in Ehrenfeld and Clewell (1987) with the following exceptions: (i) the F region has been extended to include a number of inserts in the *Eco*RI E fragment which resulted in altered surface protein production; and (ii) the E region has been extended to include an insert (NR5) with a characteristic phenotype.

The remaining regions, located further clockwise, constitute a 7-kb segment involved in the regulation of the pheromone response.

It has been proposed that two products, determined by *traA* and *traB* (Figs. 1 and 2), act in concert to negatively control the pheromone response (Ike and Clewell, 1984; Ehrenfeld and Clewell, 1987; Weaver and Clewell, 1988). Tn*917* inserts within either of these regions resulted in constitutive expression of the inducible surface proteins and cell aggregation in the absence of pheromone. Such strains also exhibit a high level of plasmid transfer in short (10 min) broth matings. These characteristics typify those of cells containing wild-type pAD1 after exposure to cAD1. Wild-type pAD1-containing cells give rise to "dry" colonies when grown on plates containing pheromone. However, *traA* mutants form dry colonies in the absence of pheromone. Colonies of *traB* mutants exhibit a "ringed" colony morphology with a dry center and become uniformly dry upon exposure to pheromone (Weaver and Clewell, 1988). The ringed colony morphology was observed in *E. faecalis* strain OG1X, but not in the unrelated host, FA2-2, where colonies appeared normal (as if uninduced) in the absence of pheromone (Ike and Clewell, 1984). Constitutive aggregation was also observed in a subset of Tn*917* insertions within the C region (Fig. 2). Colonies of these mutants produced a semidry colony morphology in OG1X more closely resembling that of *traA* derivatives. Plasmid DNA transferred constitutively, but the cells produced detectably elevated amounts of only one surface protein, AD74, in the absence of pheromone. C-region mutants could be further induced, with respect to production of surface proteins, by addition of pheromone (Weaver and Clewell, 1988). Interestingly, the A3 deletion (Fig. 2) allows production of pheromone and removes a portion of the *traB* gene and nearly all of the C region, yet no single insert in either region interferes with pheromone "shutdown." This suggests that two or more gene products may cooperate to shut down pheromone production.

The product of the *traA* gene may also be involved in transduction of the pheromone induction signal. Western blot analyses revealed that, while *traA* mutants produced all of the normally inducible surface proteins constitutively, they were produced at levels lower (10–50% depending on whether FA2-2 or OG1X was used as a host) than observed in induced cells containing the wild-type plas-

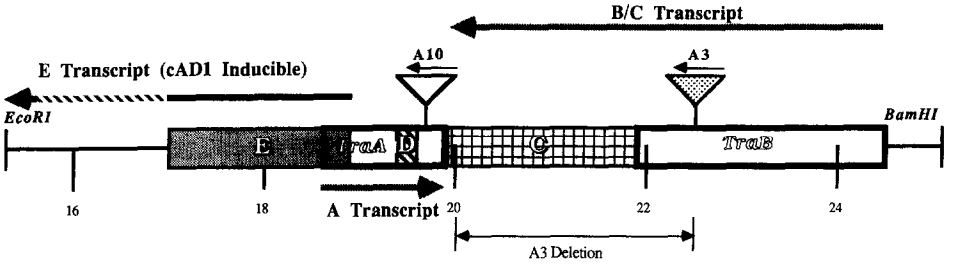

FIG. 2. Transcriptional map of the pAD1 pheromone-response control region. Direction and extent of transcripts were determined on the basis of pheotypes of a number of Tn*917* (Ike and Clewell, 1984; Ehrenfeld and Clewell, 1987) and Tn*917lac* (Weaver and Clewell, 1988) inserts and the transcription properties of *lacZ* transcriptional fusions within each region. The stipled arrows indicate the low-level constitutive expression of *lacZ* fusions in the *traA*, *traB*, and C regions compared to the high-level pheromone inducible production of β-galactosidase from E-region fusions. The cross-hatching of the E-region transcript represents uncertainty as to the terminus of this transcript. It should be noted that the transcripts indicated represent a minimum number of transcripts based on similar transcription properties of groups of fusions. It is certainly possible that one or all of the transcripts shown will be further subdivided as more information becomes available. Insert A10 and the A3 deletion are included for reference. This figure is similar to Fig. 2 of Weaver and Clewell (1988) except that the E region has been extended (see legend to Fig. 1) and the previously mapped D region is included for reference. In addition, it was found that the orientation of the A3 deletion with respect to the plasmid *Eco*RI and *Bam*HI sites was erroneously assigned (K. Weaver, unpublished results). The orientation and position of the deletion are corrected here.

mid (Weaver and Clewell, 1988; and unpublished data). The levels of these proteins in *traA* mutants could not be increased by the addition of pheromone, indicating that their expression was insensitive to the pheromone. In addition, several Tn*917* inserts in *traA* have been isolated which constitutively expressed surface proteins at a lower level than other *traA* mutants, making their failure to respond to pheromone more apparent. For example, mutants A10 (Fig. 2) and pAM727 and pAM728 (Tn*917* insertions in *traA;* Ike and Clewell, 1984) aggregated constitutively in OG1X but failed to aggregate in FA2-2 in the presence or absence of pheromone. In addition, A10 produced significantly decreased amounts of the inducible surface proteins in OG1X when compared to other *traA* mutants and undetectable amounts in FA2-2. In neither case could the amount of inducible surface proteins be increased by exposure to pheromone. Similarly, pAM727 and pAM728 showed a constitutive, but low-level transfer of plasmid DNA from a FA2-2 donor that could not be increased by the addition of pheromone. It is possible that these independently isolated mutants define the same phenotype; both were partially derepressed in the absence of pheromone, but did not respond to the presence of pheromone. Three other mutants have also been isolated near or within *traA* in a region designated D (Ehrenfeld and Clewell, 1987). Two of these three inserts failed to aggregate in response to pheromone while the third was capable of aggregation, but none of the three were capable of plasmid transfer in liquid media. Further investigation of these mutants may help to determine the role of the *traA* product in pheromone signal sensing.

E-region inserts block all aspects of the pheromone response, as derivatives failed to aggregate, produce surface proteins, or transfer plasmid DNA—even in filter matings (Ehrenfeld and Clewell, 1987). LacZ transcriptional fusions within the E region, generated using Tn*917lac* (Youngman, 1987), were shown to be inducible, clearly indicating that the E-region product(s) is not necessary for pheromone sensing (Weaver and Clewell, 1988). Thus, the related determinant(s) may include a positive regulator necessary for expression of products (e.g., in regions F, G, and H) needed for aggregation and DNA transfer. The E region may also contain the beginning of an operon running counterclockwise. It is evident that there must be several promoters in the large segment that include F, G, and H, since insertions there do not necessarily have a polar effect on expression. For example, insertions in the F region, which interfere with the aggregation response, do not seem to affect transfer functions encoded in the H region.

Analyses of fusions within the regulatory region revealed at least three transcripts on the basis of transcriptional direction and β-galactosidase production (Weaver and Clewell, 1988; Fig. 2). The E transcript is transcribed in a leftward or counterclockwise direction, and fusions within this region are inducible, as noted above. The *C/traB* and *traA* transcripts are produced constitutively at equivalent levels but are transcribed in opposite directions.

ANALYSIS OF pCF10

Transposon mutagenesis has also been used to determine the organization of pheromone response-related genes in pCF10 (Christie and Dunny, 1986). In this plasmid a central region has been identified and appears to produce two separate products, encoded within regions designated R130 and R150, which function as positive regulators of two different pheromone-inducible surface antigens, one involved in surface exclusion, Tra130, and the other in aggregation, Tra150. The structural genes for these surface antigens are located to one side of the putative positive regulators (Christie *et al.,* 1988). On the opposite side of R130 and R150, is a region, designated *tra1–tra2–tra3,* within which at least one negatively regulating product is determined. In addition, inserts in the different negative regulatory loci resulted in qualitatively different constitutive aggregation characteristics (Christie and Dunny, 1986). This arrangement is similar to the organization of pAD1, in which the negative

regulatory genes, *traA* and *traB,* and genes which affect the production of various surface proteins (the F region) are located on opposite sides of a putative positive regulatory region (the E region). Some differences in gene arrangement are apparent between the two plasmids, however. For instance, with the possible exception of a single locus identified by three Tn*917* inserts, no region of pCF10 has yet been identified which is absolutely required for induction of all aspects of the pheromone response. In addition, inserts in several regions on the opposite side of R130 and R150 from *tra1–tra2–tra3* have been isolated which resulted in constitutive aggregation (Christie and Dunny, 1986). It will be interesting to compare the genetic organization of pAD1 and pCF1 as work progresses.

CONCLUDING REMARKS

The fact that plasmid-free strains of *E. faecalis* excrete multiple peptides which serve as mating signals relating to a variety of plasmids perhaps never previously encountered suggests that production of these substances may have preceded the evolution of the related conjugative systems. The plasmid systems may have evolved in such a way as to take advantage of these extracellular compounds as mating signals. The question then arises as to the nature of the original, and perhaps continuing, function of these peptides. In this regard, it is interesting that an activity similar to that of the pheromone cAM373 (Clewell *et al.,* 1985) is also produced by essentially all strains of coagulase positive staphyococci (i.e., *Staphylococcus aureus*), but not by coagulase negative staphylococci (e.g., *S. epidermidis*); the activity is also produced by about 15% of *Streptococcus sanguis* strains. There is no evidence that such activities act as pheromones in these species.

It is surprising that in the 10 years since sex pheromone-induced plasmid transfer was discovered in *E. faecalis,* such mating signals have still not been identified in other species of bacteria. The closest thing to sex pheromones may be the competence factors associated with transformation phenomena of *Streptococcus pneumoniae* and *Streptococcus sanguis* (Tomasz, 1969; Lacks, 1977). These extracellular substances seem to facilitate a synchronous appearance of the competent state of a given culture. However, in nature it is conceivable that transformational recombination between different strains could be influenced by these compounds. In such cases these factors might, in a sense, be viewed as sex pheromones.

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