MiniReview

Molecular biology of the capsular genes of *Streptococcus pneumoniae*

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Abstract

The polysaccharide capsule of *Streptococcus pneumoniae* is the main virulence factor of this microorganism. Although the study of the genes responsible for the synthesis of the pneumococcal capsule enabled genetic and molecular analysis, the precise structure, organization, and functioning of these genes have only been investigated very recently. The genes implicated in the production of the type 3 capsule have been sequenced, expressed and their corresponding products biochemically characterized. In addition, partial information on the genes responsible for the biosynthesis of the capsules of pneumococcal types 1, 14 or 19F is currently available.

Keywords: *Streptococcus pneumoniae*; Pneumococcus; Capsular polysaccharide; Genetic transformation

1. Introduction

Bacterial surface polysaccharides play an important role in determining the virulence of many Gram-positive and Gram-negative bacteria. Much of the interest in the genetics and control of synthesis of these polysaccharides stems from this fact. The main function of the capsule is to prevent the ability of polymorphonuclear leukocytes from engulfing and digesting bacteria. Although *Streptococcus pneumoniae* produces many virulence factors [1], as early as in 1928, Griffith reported that unencapsulated pneumococcal variants were avirulent [2], indeed loss of the capsule is accompanied by a 10⁶-fold reduction of the virulence of *S. pneumoniae*. Unencapsulated pneumococci are readily phagocytosed when added to a suspension of leukocytes in normal serum, whereas mucoid, encapsulated organisms are resistant to phagocytosis and multiply rapidly. A quantitative relationship between the amount of type-specific polysaccharide and virulence has been found, although the chemical composition of the capsule as well as the cellular background in which the capsule is produced also appear to play an important role in virulence [3].

Transformation of the pneumococcal types was first described by Griffith in 1928 [2] who inoculated mice subcutaneously with a mixture of live unencapsulated pneumococci and a vaccine of heat-killed encapsulated pneumococci. The mice developed an infection due to encapsulated pneumococci of the same type as the strain from which the vaccine had been prepared. Although it was shown later that type transformation could occur in vitro and could be induced by a cell-free extract of the donor organ-

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isms, it was not until 1944 that Avery and co-workers reported that DNA was responsible for inducing genetic transformations [4]. Most of the results on pneumococcal transformation reported before 1960 can be found in a comprehensive, classic review [5]. In vivo transformation events involving capsular genes have been observed [6], and serotype transformation has been suggested as the most likely explanation for the identification of clinical isolates of *S. pneumoniae* that differ only in the capsular polysaccharide [7].

Mäkelä and Stocker [8] reviewed the classic studies which provided evidence indicating that the genes responsible for capsular polysaccharide biosynthesis were closely linked in the pneumococcal chromosome and could be transferred as a unit during transformation. Since transformation of pneumococci to heterologous type (inter-type transformation) implies the substitution of the capsular polysaccharide of the recipient cell by that of the donor type, this kind of transformation event should involve the exchange of large pieces of DNA containing the genes responsible for the synthesis of the sugar components of the corresponding capsular polysaccharide as well as the transferase(s) implicated in the polymerization step. A significant variation of this general rule has been reported. When unencapsulated type 3 mutants (S3−) deficient in UDP-glucose dehydrogenase (UDP-GlcDH) activity were transformed with DNA prepared from a capsulated type 1 strain (S1+), a minority of the transformants were of ‘binary type’, that is, they reacted with both anti-S3 and anti-S1 sera and produced both kinds of capsule [9]. Subsequent studies indicated that binary transformants retain the recipient type 3 capsular genes and also possess the capsular gene cluster of the donor DNA which includes a gene encoding the UDP-GlcDH. In addition, transformation experiments using DNA prepared from binary cells showed that the supernumerary capsular genome was inserted in the chromosome of the recipient strain in a region different from the usual capsular-polysaccharide-determining one [8]. Binary transformants were isolated not only from S3 × S1 crosses but also in transformation experiments implicating DNAs prepared from pneumococci of other serotypes that also contain uronic acids.

Although initial studies on the genetics of capsular polysaccharides were done in *S. pneumoniae*, most of our current knowledge on the organization of genes responsible for the synthesis of capsular polysaccharides comes from studies carried out in Gram-negative bacteria [10]. In *Escherichia coli* there is a gene cluster containing three distinct regions implicated in the expression of type II capsular polysaccharide. Region 1 is needed for the transport of mature, lipid-linked polysaccharide across the outer membrane and its assembly into a capsule. Region 2 is serotype-specific, encodes the enzymes responsible for the synthesis and polymerization of the polysaccharide, and its length is directly proportional to the chemical complexity of the capsule. Region 3 contains two genes (*kpsM* and *kpsT*) that are involved in the translocation of the polysaccharide through the inner membrane. Serotype-specific genes are flanked by genes common to all serotypes which appear to be involved in common functions. Nothing is known about the cellular functions implicated in capsule transport and assembly in Gram-positive bacteria.

### 2. Cloning and sequence determination of the *S. pneumoniae* capsular genes

Ninety different pneumococcal capsular polysaccharides have been described so far [11] and although the chemical composition of most of them has been established [12], only four serotypes have been studied at the molecular level. Two groups have investigated serotype 3 since it is the type most frequently isolated from clinical samples obtained from adults, has a relatively simple chemical structure (Table 1), and is easily identified on plates. In addition, a great number of genetic and biochemical studies were carried out with *S. pneumoniae* strains belonging to this serotype until the early 1970s [8]. Other groups have studied the genes coding either for type 19F capsular polysaccharide, which is one of the most frequent causes of invasive disease in children, or for type 14, for which some biochemical knowledge accumulated during the 1960s [13]. We are also currently studying the type 1 capsular genes.

Since many of the natural, encapsulated pneumococcal isolates are poorly transformable, apparently due to a defect involving the synthesis and/or export of the activator protein (competence factor), we de-
decided to construct isogenic, highly transformable S3 strains that could be used as recipients in transformation experiments [14] and should differ from the parental S3 strain by a single mutation [15]. Other researchers have employed alternative methods for cloning purposes, i.e., plasmid insertion and rescue [16,17], inverse polymerase chain reaction (PCR) amplification [17], or a combination of hybridization of pneumococcal DNA libraries with capsular genes from S. agalactiae and transposon mutagenesis [18].

2.1. Nomenclature of pneumococcal capsular genes

Bernheimer and Wermundsen [19] first designated the capsular genes of types 1, 2, 3, etc., as cap1, cap2, cap3, etc. Nevertheless, several authors have recently named the capsular genes of S. pneumoniae as cps (for capsular polysaccharide synthesis). Although priority rule for gene designation must be preserved, for the sake of clarity, we have maintained the nomenclature for pneumococcal capsular genes used in the original works.

2.2. DNA regions common to pneumococcal isolates of several serotypes

Fig. 1 shows the organization of the capsular genes of several pneumococcal types using the sequences available in the data banks and our own unpublished results (as for type 1). DNA hybridization was used to differentiate between regions conserved in most (if not all) serotypes (represented by green shading in Fig. 1) and those regions that appeared to be type-specific or to be present only in related serotypes. The open reading frame (ORF) located at the extreme of the region analyzed corresponds to a gene designated dexB since the deduced amino acid sequence of this gene shows 65% identity with a glucan 1,6-α-glucosidase of S. mutans and S. equisimilis. Inactivation of dexB by insertion-duplication mutagenesis indicated that this gene is not involved in capsule formation either in type 19F [17] or in type 3 [20] strains. Immediately downstream of dexB there is a region of about 1 kb (represented by a thin, pink line in Fig. 1) that appears to be highly polymorphic among different serotypes [20] as well as among various isolates of the same serotype [21]. It should be emphasized that a close examination of this region from types 1, 3, and 19F revealed the presence of two incomplete ORFs (not shown). The one closest to dexB is oriented as this gene and seems to represent part of a new aliA homologue [22] whereas the other reading frame, which is oriented oppositely to dexB, showed a noticeable similarity with some genes coding for eukaryotic transposase-like sequences (J.-P. Claverys, personal communication). It should be mentioned that AliA is a member of a family of membrane-bound lipoproteins that participate in oligopeptide transport in S. pneumoniae and that are functionally equivalent to the periplasmic substrate-binding component of ABC transporters of Gram-negative bacteria [22]. A characteristic feature of this region appears to be the presence of insertion elements (IS), namely, IS1202 [23] and IS1167 in types 19F and 1, respectively. In the case of type 1 (unpublished results), the IS1167 copy is interrupted by two frameshift mutations (indicated as a thin, blue arrow in Fig. 1) instead of only one as reported by Zhou et al. [24]. In contrast, no IS has been found in this region in a type 3 clinical isolate [20].

Two ORFs (cap1A/cap1B and cps19fA/cps19fB in types 1 and 19F DNAs, respectively) appear to be conserved in all the type DNAs tested, as judged by Southern blot hybridization [17]. However, a global analysis of this region in type 3 DNA did not reveal the presence of any intact ORF in spite of the fact that comparison between the nucleotide sequence of this region and the corresponding one of type 19F showed that they were more than 95% identical [20]. An approximately 280 bp long region corresponding to the 5' end of the genes cap1A/cps19fA is deleted in type 3 DNA, and several additional, minor deletions were also reported. The predicted gene products of cps19fC and cps19fD were virtually identical (more than 93% identity) to those encoded by orf1cap1C and orf2cap1D, respectively. Very recently, the nucleotide sequence of the genes cps14BCD has been deposited with the GenBank/EMBL database (Fig. 1). The possible function of the gene products of all these ORFs is still a matter of speculation since the proposed roles are only based on sequence comparisons. It has been suggested that Cps19fA may act as a regulator of the capsular gene expression whereas Cps19fC and Cps19fD might be involved
in the export of type 19F capsular polysaccharides [17].

The DNA located downstream of the type 3-specific region contains sequences common to apparently all capsular types [16,25,26]. Sequence analysis of this region revealed the presence of only one complete ORF. The analysis of the deduced amino acid sequence (373 residues) of the product of orf3 (Fig. 1) showed that this protein has an amino-terminal sequence with the characteristics of a signal peptide suggesting that the orf3 gene product is exported across the pneumococcal membrane. Search for similarities showed that the first 200 amino acid residues are 24% and 22% identical to those of the NanA neuraminidase of S. pneumoniae, and the IgA-binding β antigen (Bag) of group B streptococci [26], respectively. No other obvious similarities were found and the function of this protein remains unknown. The 3-kb DNA region located between cap3C and orf3 contains several incomplete reading frames. Dillard et al. [25] reported the existence of a fourth type 3-specific gene that was named cps3M (represented in Fig. 1 as a thin, purple shaded arrow) and suggested that it was homologous to phosphoglucomutases from several bacterial species although this assumption is not completely supported by the analysis of the sequence located immediately downstream of cap3C. Nevertheless, the deduced amino acid sequence of this reading frame did contain a typical phosphoglucomutase/phosphomannomutase phosphoserine signature. Hybridization experiments confirmed that this reading frame is type 3-specific although dispensable for capsule biosynthesis [25]. This is in agreement with our finding that the corresponding sequence in the clinical strain 406 (S3+) is interrupted by an incomplete reading frame that is 55% identical to the IS1167 element [24] (represented in Fig. 1 as a thin, blue arrow). This insertion element is also present in the corresponding position of type 1 DNA (Fig. 1). Finally, the 1.1-kb DNA fragment located between the IS fragment and orf3 corresponds to the aliA gene of S. pneumoniae encoding an oligopeptide permease [22] although the reading frame of this gene in strain 406 is truncated at its 5' end and contains two frameshift mutations [26]. The existence of a deleted form of the aliA gene in this region of type 3 strains had been reported previously [25] whereas preliminary data indicate that type 19F [27] and type 1 (unpublished) strains contain a complete copy of the aliA gene.

2.3. Sequence analysis of the type-specific capsular genes

The DNA regions located downstream of cps19F, cps14D, or cap1D contain the group 19- [17], type 14- [28] and type 1-specific genes, respectively. A noticeable difference between type 3 and the other serotype DNAs that have been studied so far is the existence in type 3 of an intergenic space of about 1100 bp between orf2 and cap3A (Fig. 1) which contains sequences common to other pneumococcal serotypes [14,25]. Sequences characteristic of a consensus σ70 promoter have been located upstream of cap3A [14]. Computer searches for sequence similarities were carried out in order to gain information on the possible role of Cps19F,E, Cps19f,F, and Cps19f,G on type 19F capsule biosynthesis and it was suggested that they are glycosyl transferases [17]. It is noteworthy that cps19F,E is 98% identical to cps14E whereas cps14F showed no similarity to any known gene [18]. This observation provides evidence on genes common to several capsular types that are involved in the formation of type-specific capsule, i.e., the genes cps19f,F and cps19f,G appear to be also present in types 19B and 19C DNAs but not in type 19A DNA [17]. On the other hand, it has been found that Cap1K is 61% identical (77% similar) to Cap3A [29].

Several spontaneous S3− isolates of S. pneumoniae were characterized as cap3A mutants and three different mutations have been sequenced [15]. More recently, point as well as insertion mutations affecting cpsD (cap3A) have been reported by Dillard et al. [25] and all of them also conferred an unencapsulated phenotype. The 45-kDa Cap3A protein was suggested to correspond to the type 3-specific UDP-GlcDH of S. pneumoniae since it is homologous (57% identity and 74% similarity) to the UDP-GlcDH (HasB) of S. pyogenes [30]. The cap3A+ allele restored capsular synthesis in mutants previously characterized as deficient in UDP-GlcDH which supports the conclusion that cap3A codes for UDP-GlcDH [15,16]. The cap3B and cap3C genes encode proteins of approximately 49 and 34 kDa, respectively [20,25]. Sequence comparison showed
that Cap3B is similar to several polysaccharide synthases previously characterized, and in vitro capsule synthesis assays suggested that Cap3B was the type 3 capsule polysaccharide synthase [25]. Computer searches revealed that Cap3C is most probably a UDP-Glc pyrophosphorylase, the enzyme responsible for the synthesis of UDP-Glc from Glc-1-P and UTP. The cap3C gene was capable of reverting an E. coli galU mutant which confirmed that Cap3C is a UDP-Glc uridylyltransferase [20].

3. Functional organization of the capsular genes

The capsular genes in type 19F pneumococci are arranged as an operon (cps19fABCDEFG) and Guidolin et al. [17] reported that insertion-duplication mutagenesis of any of the six potential ORFs produced transformants that exhibited an unencapsulated phenotype. However, a definite conclusion on the functional organization of the type 19F capsular cluster still needs the characterization of in-frame deletions or point mutations rather than insertion mutations that most probably produce polar effects. Direct molecular evidence of the involvement of certain genes in capsule formation of type 3 pneumococci has been recently achieved by the use of point mutations, and we have determined the sequence defect of three different cap3A mutants [15] which confirmed previous results obtained by biochemical and genetic means [8]. In addition, a transcriptional analysis has been helpful to determine the functional organization of the type 3 capsular genes [20]. Northern blot and primer extension analyses showed a 5.8-kb mRNA that includes at least cap3A, B, and C and demonstrated that transcription of type 3-specific genes starts 59 nucleotides upstream of the ATG initiation codon of the cap3A gene. The length of the capsular transcript is sufficient to include the truncated ORF located immediately downstream of cap3C (Fig. 1) which should represent the fourth gene of the cap3 operon (see above). In addition, our results indicated that the genes located between dexB and cap3A are not apparently transcribed. On the other hand, insertion-duplication experiments showed that cap3C is not required for type 3 capsular biosynthesis [20,25] suggesting that the function of Cap3C is compensated for by another enzyme.

Recently, Watson et al. [31] reported that a region of the pneumococcal chromosome located downstream of the hytA gene encoding the major autolysin (an N-acetylmuramoyl-l-alanine amidase) was essential for encapsulation. This conclusion was reached by sequencing a region of DNA flanking the insertion of transposon Tn916, an insertion that resulted in a lack of type 3 capsule expression. Since the Tn916 insertion site in the mutant strain was located far away from the type 3-specific genes it was suggested that this region might be involved in some kind of regulation of capsule biosynthesis. Taking into account that the chromosomal region claimed to be essential for capsule formation is deleted in strain M31 (an S2- strain isolated some years ago in our laboratory) we transformed this strain to the S3+ phenotype using DNA from the clinical isolate 406. Pulsed-field gel electrophoresis and Southern
4. Biochemical characterization of gene products

Kolkman and co-workers [18] using insertion-duplication mutagenesis demonstrated that both \(\text{cps}14E\) and \(\text{cps}14F\) are involved in capsular synthesis by bladder

blot hybridizations revealed that the encapsulated transformant of M31 still harbored a deletion identical to that of the parental strain demonstrating that the region that lies just 3' of the \(\text{lytA}\) gene is not essential for the synthesis of type 3 capsule [32].
of *S. pneumoniae* (Fig. 1). In addition, membranes prepared either from wild-type pneumococcal cells or from the *cpsl4F* mutant incorporated [14C]Glc from radioactively labeled UDP-Glc whereas those prepared from a *cpsl4E* mutant did not, indicating that *cpsl4E* encodes a glycosyl transferase. More recently, evidence suggesting that Cps14F and Cps14G function together as a galactosyl transferase has been obtained [28]. On the other hand, the characterization of the capsular polysaccharide biosynthesis locus of *S. pneumoniae* type 19F has been recently carried out [27].

As reported above, several lines of evidence had suggested that *cap3A* coded for a UDP-GlcDH. Nevertheless, direct biochemical evidence has only been provided very recently. Repeated attempts to clone the entire *cap3A* gene in *E. coli* together with its own promoter were unsuccessful and only deleted recombinant plasmids were obtained suggesting that overproduction of this enzyme might be toxic for the host cells. In order to clone *cap3A* in the absence of its own promoter, this gene was first PCR amplified and then cloned under the control of the *φ10* promoter (a T7 RNA polymerase-inducible promoter). Upon addition of isopropyl-β-D-thiogalactopyranoside (IPTG), the *cap3A* gene was overexpressed in *E. coli* as a 46-kDa protein [33] which is in agreement with the *M*<sub>r</sub> (44,646) deduced from the nucleotide sequence of *cap3A*. The identity of this protein with Cap3A was ascertained by the determination of the N-terminal amino acid sequence. Spectrophotometric determinations and high-performance liquid chromatography analysis of the reaction products demonstrated that *E. coli* extracts containing Cap3A exhibit UDP-GlcDH activity. Evidence showing that *cap1K* codes for the type 1 UDP-GlcDH has also been recently reported [29].

The *cap3B* gene was also expressed in *E. coli* under the control of the *φ10* promoter but the presence of the pLysS plasmid in the recipient *E. coli* strain appears to be essential for the successful cloning of *cap3B*. This requirement is possibly due to the fact that the lytic enzyme of the T7 bacteriophage coded by pLysS inhibits the viral RNA polymerase and thus reduces further the expression of the inducible *φ10* promoter. The Cap3B protein was expressed in *E. coli* as a 49-kDa protein upon the addition of IPTG [34]. More importantly, sonicated extracts prepared from induced cultures of *E. coli* (pTBP3) contained highly polymerized, type 3 polysaccharide. Uninduced cultures also synthesized pneumococcal polysaccharide although at a lower rate (10–25% of that found in induced cultures). These results demonstrated that Cap3B is the type 3-specific synthase of *S. pneumoniae* and that it has both UDP-Glc and UDP-GlcA transferase activities and possibly synthesizes the type 3 polysaccharide in a processive manner by monomer addition as is the case of the hyaluronan synthase (HasA) of group A streptococci [35]. On the other hand, subcellular fractionation studies revealed that as much as 40% of the type 3 pneumococcal polysaccharide synthesized was located in the periplasmic space of *E. coli*. It is still uncertain whether the mechanism for the transport of pneumococcal polysaccharide is the same as that used for the transport of the homologous *E. coli* polysaccharide. In Gram-positive bacteria, the mechanism of polysaccharide transport has not been characterized so far, although for the type III capsule of group B streptococci [36] and type 19F pneumococci [17] it has been suggested that some of the genes located upstream of the specific capsular cluster might be responsible for the transport of the intracellularly synthesized capsular polysaccharide. Nevertheless, the DNA region corresponding to the reading frames suggested to participate in the transport of the polysaccharide of group B streptococci and 19F pneumococci is not functional in type 3 pneumococci as reported elsewhere [20], strongly suggesting that another still-unknown mechanism may be responsible for the export of the capsular polysaccharide through the cytoplasmic membrane.

The *cap3B* gene was also cloned into a shuttle plasmid capable of replicating both in *E. coli* and *S. pneumoniae*, and the recombinant plasmid pLS3B was introduced by transformation into pneumococcal strains of capsular types 1, 2, 5, and 8, all of them containing hexuronic acids [12]. Quellung reaction and immunodiffusion analyses demonstrated that the corresponding transformants were of the binary type and expressed a type 3 capsule together with that of the recipient cell. These results strongly suggested that the mechanism of polysaccharide transport of the recipient cells is used for the formation of a type 3 capsule in binary transformants. Furthermore, when two S2<sup>−</sup> laboratory
derivatives of the rough strain R36A were transformed with pLS3B, the transformants expressed a type 3 capsule (but not type 2). In every case, the type 3 polysaccharide produced was of high $M_r$ [33]. In conclusion, provided that the corresponding sugar nucleotide precursors (UDP-Glc and UDP-GlcA) are present, cap3B appears to be the only gene required to direct type 3 polysaccharide biosynthesis in pneumococcal strains or in *E. coli*.

5. Concluding remarks

Classic studies using genetic transformation allowed the coining of the term ‘capsular genome’ meaning the closely linked genes controlling the production of capsular polysaccharide [8]. Inter-type transformation takes place when the donor DNA displaces the resident capsular genome and it was assumed that this interchange was mediated by homologous sequences flanking the type-specific gene cluster. Our current knowledge of the organization of capsular genes at the molecular level, although limited, fully confirms those early interpretations. In types 19F, 3, and 1 (and possibly also in type 14) the genes responsible for the synthesis of the corresponding activated sugars and the specific glycosyl transferases (synthases) are clustered together in the *S. pneumoniae* chromosome and located close to the genes encoding the penicillin-binding proteins PBP1a and PBP2x [15]. The putative functions attributed to different gene products have been deduced mostly from sequence comparison except for genes cap3ABC, cps14E, and cap1K where direct experimental evidence of their role in capsule biosynthesis has been recently reported. Moreover, in the case of type 3, our results fully confirmed the previously suggested biochemical pathway for the formation of this capsular polysaccharide. Thus, the transferase encoded by cap3C (or an analogous gene) will convert Glc-1-P into UDP-Glc. Then, the Cap3A UDP-GlcDH will lead to the formation of UDP-GlcA, and finally, the Cap3B synthase will link the activated monosaccharides to provide the formation of type 3 polysaccharide. It is of particular interest that expression of only one gene (cap3B) was required for the synthesis of high molecular size, type 3 polysaccharide in *E. coli* and *S. pneumoniae* strains that produce UDP-GlcA. It is also noteworthy that approximately 50% of the pneumococcal type 3 polysaccharide synthesized in *E. coli* appears in the periplasmic space, although the mechanism for this transport remains to be elucidated. A type 3 capsule was assembled both in capsulated and in unencapsulated pneumococcal strains when transformed with a plasmid expressing the Cap3B synthase and we postulate that the mechanism of polysaccharide transport of the recipient cell is used for the formation of a type 3 capsule.

Sequence comparison suggested that some of the genes located upstream of the type-specific cluster might be implicated in polysaccharide transport and/or assembly, as has been shown to occur in Gram-negative microorganisms [10]. In types 1 and 19F DNAs, the capsule-specific genes are apparently organized forming an operon that also includes four genes located upstream. However, this is not the case in the DNA of type 3 pneumococcal strains where it has been demonstrated that the promoter of the capsule-specific genes is located immediately upstream of cap3A. In addition, sequence determination and Northern blot analysis strongly suggested that the DNA sequences located upstream of the type 3-specific genes represent pseudogenes that are not transcribed. It is yet to be determined whether the common genes discussed in this review are actually required for capsule formation or, alternatively, the transport of polysaccharide and its assembly into a capsule should be driven by other still-unknown genes. Alternatively, we cannot exclude the possibility that diverse functional organizations of the capsular genes may exist in different pneumococcal serotypes.

There is increasing evidence suggesting that capsular biosynthetic genes can be transferred among different clinical isolates of *S. pneumoniae* [7]. It is tempting to speculate that the IS-like elements located at one (type 3 and 19F) or both sides (type 1) of the capsular genes might have been involved in horizontal transfer of the pneumococcal capsular genome. Furthermore, Yother has noted [37] that the DNA sequences located between orf2 and cap3A correspond to part of genes similar to some prokaryotic transposable elements. Interestingly, it has been recently reported that the type 1 capsule-encoding genes (cap1) of *Staphylococcus aureus* are located in
a discrete genetic element of about 34 kb terminated, at one end, by an IS-like element [38], and it is also well established that the type b capsular genes of Haemophilus influenzae are located in a 17-kb compound transposon [39].

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