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Sialic acid, periodontal pathogens and *Tannerella forsythia*: stick around and enjoy the feast!

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Abstract

Periodontal pathogens, like any other human commensal or pathogenic bacterium, must possess both the ability to acquire the necessary growth factors but also the means to adhere to surfaces or reside and survive in their environmental niche. Recent evidence has suggested that sialic acid containing host molecules may provide both of these requirements *in vivo* for several periodontal pathogens but most notably for the red complex organism *Tannerella forsythia.* Several other periodontal pathogens also possess sialic acid scavenging enzymes – sialidases, which can also expose adhesive epitopes, but might also act as adhesins in their own right. In addition recent experimental work coupled with the release of several genome sequences has revealed that periodontal bacteria have a range of sialic acid uptake and utilisation systems while others may also use sialic acid as a cloaking device on their surface to mimic host and avoid immune recognition. This review will focus on these systems in a range of periodontal bacteria with a focus on *T. forsythia.*

Sialic acid as a growth factor or carbon source

An increasing number of human pathogens are being uncovered that have the ability to use sialic acid as a growth factor or sole carbon source (Vimr ref, Severi ref, others). These now include representatives of several bacterial genera (including *Neisseria (ref), Haemophilus (ref), Bacteroides* (ref), *Fusobacteria (ref)* and *Streptococci ref)* that inhabit a range of biological niches within the human body from the oral cavity through the respiratory system and into the gastrointestinal and urinary tracts, although none have been found in free-living bacterial species to date.

While sialic acid seems an obvious source of carbon for bacterial pathogens and other human and mammalian dwelling bacteria since it is present on the surface of glycoproteins, gangliosides and sphingolipids (ref), its role in the biology and pathogenesis of periodontal pathogens is only now coming to light. The discovery that the nutritionally fastidious periodontal pathogen *Tannerella forsythia* is able to substitute its requirement for N-acetyl muramic acid (MurNAc) (Wyss), a building block used to produce cell wall peptidoglycans,

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with sialic acid in biofilm culture (ref) was not only surprising but also suggested that *in vivo* it may actually be more adaptable than its fastidious laboratory growth requirements suggest. Its ability to utilise sialic acid is reliant on a large *nan* gene cluster located over an 16kb section of its genome (Fig 1). This cluster contains all the genes required for sialic acid catabolism *(nanA, nanE)*, using a putative pathway that is most related to that of the Gastrointestinal (GI) anaerobe *Bacteroides fragilis* (Brigham), and uptake across both inner *(nanT)* and outer *(nanOU)* membranes plus several auxillary genes which most likely play a role in scavenging sialic acid from the environment *(nanS, hexA, nanH)*. Indeed a mutation in the *nanH* gene abrogates growth of this organism with siallylactose as the sole sialic acid source in biofilm culture (Roy new paper). This operon bears homology both at the sequence level but also at the genome organisation with related human dwelling GI anaerobes such as *B. fragilis (BF1711-1720, BF1806-1809)* and *Parabacteroides distastonis (Figure 1)* and represents a new departure from the *E. coli* paradigm pathway for sialic acid utilisation that dictates the requirement of a neuraminate lyase (NanA), N-acetylmannosamine epimerase (NanE) and an N-acetylmannosamine kinase (NanK) in an operon alongside relevant regulatory, accessory (NanM mutarotase, NanS neuraminate acetyl esterase and transcriptional regulator genes (NanR) (Vimr, Severi) (Fig.1). In *E. coli* and *Haemophilus influenzae* NanK is required for phosphorylation of N-acetylmannosamine (ManNac) to ManNac-6P before conversion into N-acetylglucosamine-6-phosphate (NAG-6P). However, in *B. fragilis* and probably in *T.* forsythia NanE is capable of converting ManNac to N-acetyl glucosamine, which is then phosphorylated by a hexokinase called RokA *(Tf1997* in TF) before being processed by the rest of the pathway (Brigham et al., Roy et al.). Evidence discussed below also suggests that this group of organisms have also adapted a TonB dependent transport module to deal with sialic acid.

In view of the ability of *T. forsythia* in particular to use sialic acid one asks the question why and how can it substitute for NAM? And strikingly, why only in a biofilm? However, at present while one can only speculate why this is the case, it is worth noting that *in vivo* this organism would probably be present as part of the subgingival plaque biofilm where the ability to utilize sialic acid must confer a competitive advantage for nutrition. We have evidence that *nan* operon gene expression is not only induced in biofilm but also that several of these genes are induced in the presence of sialic acid (Ref Sumi Thesis). In the absence of a putative regulator in this region of the chromosome the route for this regulation is unclear. The ability of this notoriously fastidious bacterium to substitute sialic acid for NAM at all is almost as intriguing as its ability to utilize NAM in the first place (Wyss). Our hypothesis is that *T. forsythia* might use sialic acid as a means to produce N-acetylmuramic acid (NAM, MurNAc) from N-acetylglucosamine but in a presumably inefficient manner since the amount of sialic acid that supports *T. forsythia* growth in biofilm (6mM) is far in excess of the 170μM NAM that is equivalent (Roy et al). In *E. coli*, NagE (GlcNAc-specific phosphotransferase enzyme) (Plumbridge, 2009) and NagZ (N-acetylglucosaminidase) (Dahl *et al.*, 2004) are responsible for converting N-acetylglucosamine (NAG) to N-acetylmuramic acid (NAM) while *Bacillus subtilis* uses NagE and MurP (MurNAc-specific phosphotransferase system (PTS)) to yield NAM that it converts into MurNAc-6-phosphate using the NagZ orthologue MurQ (MurAc-6-phosphate-esterase) (Litzinger *et al.*, 2010). However, *T. forsythia* does not contain any homologues of NagE, MurP or MurQ and also

lacks the two step NAM synthesis pathway which includes UDP-N-acetylglucosamine-enolpyruvate transferase and UDP-enolpyruvate reductase (Kolenbrander book: A. Sharma, Genome functions of *Tannerella forsythia* in bacterial communities. in: P.E. Kolenbrander, (ed.) Oral Microbial Communities: Genome inquiry and interspecies communication, American Society for Microbiology, Washington, D. C., 2011.). Even more intriguing is how exogenous NAM is transported and utilized by *T. forsythia* in the absence of a putative PTS-like amino sugar uptake system. This suggest that the NAM (and sialic) metabolic pathways in *T. forsythia* are likely to utilize completely novel pathways and enzymes for sugar transport and utilization of these sugars. The identification and characterization of these pathways will be a focus of our laboratories and others in the near future.

Other periodontal pathogens

While several studies on the periodontal pathogen *Porphyromonas gingivalis* have highlighted a role for sialic acid in adhesion to human cells – refs from dunhill grant, there is no evidence that it utilises sialic acid as a growth substrate nor contains any catabolic genes in its genome sequence. A similar story also seems to hold true for the fellow red-complex periodontal pathogen *Treponema denticola* with both possessing at least one putative sialidase encoding gene (genome and Fletcher).

Among other periodontal bacteria the presence of sialic acid catabolic and scavenging genes is varied. For example, the Gram-negative periodontal pathogen Aggregatibacter actinomyceteconcomitans (Aa contains a putative *nan* operon, while the orange complex bridging organism *Fusobacterium nucleatum* harbours a full sialic acid utilisation operon comparable to that of *H. influenzae* with catabolic, inner membrane transport (TRAP type, see below) and regulatory genes clustered together (Figure 1). In addition *F. nucleatum* has previously been shown to be able to utlilise Sialic acid as a sole carbon source - REF. However, the closely related oral species *Fusobacterium nucleatum subsp polymorphum* has no catabolic genes but does contain an *lst* operon that is potentially involved in LPS sialylation (see below). These observations are intriguing given that *F. polymorphum* is known to form synergistic biofilms with *T. forsythia* via co-aggregation dependent mechanisms- implying that *T. forsythia* may adhere to the *F. polymorphum* surface and scavenge its sialic acid for growth (Ashu synergy paper. In this regard, NanH sialidase is likely to be involved in binding to and cleaving *F. polymorphum* surface sialic acid residues. In support of this we have preliminary data suggesting that *F. polymorphum* coaggregates less readily with our *T. forsythia nanH* mutant than the wild-type and that its LPS banding pattern is altered after sialidase treatment *in vitro* (unpublished data). Thus it may be that sialic acid also plays a role in nutritional and physical interactions between bacteria known to cohabit within subgingival biofilm. These interactions may also contribute to fitness *in vivo* as removal of sialic acid from the lipooligosaccharide of *H. Influenzae* and *Neisseria meningitidis* by the NanA sialidase of *Streptococcus pneumoniae* has been suggested to contribute to survival in the respiratory tract during co-infections (Shaknovich et al., 2002).

Diversity of membrane transport systems in periodontal bacteria

Despite the obvious requirement for largely similar biochemical pathways for sialic acid utilisation in pathogenic bacteria, i.e. a mechanism to breakdown sialic acid and assimilate

into biomass, there is much more diversity in the mechanisms by which sialic is transported from the extracellular to intracellular environments.

Transit of sialic acid across the inner membrane has been studied in some detail with the identification of dedicated uptake systems that fall into two main categories: 1) Major Facilitator permeases, commonly annotated as NanT (Vimr, Kalivoda, Deszo, & Steenbergen, 2004) and 2) the *siaPQM* Tripartite ATP-independent periplasmic (TRAP) transporters (Steenbergen *et al.*, 2005) (Figure 2). Recently a third type of sialic acid transporter was discovered in *Salmonella typhimurium* which is predicted to be present in a range of pathogenic bacteria and is a member of the sodium solute symporter (SSS) family (Severi *et al.*, 2010), while the SatABCD system seems to be limited to *Haemophilus ducreyi* (ref). Our published work has established that the NanT type permease of *T. forsythia* is functional upon transplantation into *E. coli* (ref). Bioinformatic searches of completed genomes reveals that in addition to sharing similar sialic acid catabolic pathways with enteric *Bacteroides* species, the sialic acid transport systems also seem to be related. Both possess a NanT type permease and notably the novel TonB-dependent NanOU outer membrane transporters also identified in *T. forsythia* (Figure 1). The other major type of sialic acid inner membrane transport system is of the TRAP family, the mechanisms of which have been revealed in classic biochemical studies by several groups (reviewed by Thomas…). It is in fact this TRAP type transporter that is present in *F. nucleatum* and *Aa* (Figure 1,2).

In contrast to the inner membrane transport systems, only two sialic acid specific outer membrane transporters have been identified to date. The first was the NanC sialic acid-specific outer membrane porin from *E. coli* K-12, which is essential for growth on Neu5Ac when the general porins, OmpF, OmpC are not expressed (Condemine *et al.*, 2005). In the genomes of periodontal bacteria sequenced to date, NanC homologues have not been identified thereby leading to the assumption that a general porin may perform this function in these organisms (Figure 2). Our recent work identified the functionality of a second type of outer membrane sialic acid transport system in the *T. forsythia* sialic acid operon, encoded by the genes NanO and NanU *(TF0033* and *TF0034)*, that are able to complement sialic acid growth defects in an *E. coli* strain devoid of sialic acid transport across its outer membrane (Roy). The protein encoded by *nanO* is a member of the TonB-dependent receptor family, a class of protein that is often involved in small molecule transport in a mechanism that is energised by the TonB-ExbB-ExbD (TBDR) protein complex (unpublished Roy). Such complexes are typically involved in iron transport but are becoming increasingly recognised as having a role in sugar transport in a range of organisms (3,44,47) and in signal transduction and transcription via ECF-type sigma factors (19). In fact, *T. forsythia* contains over 60 TBDRs in a genome of 3.4 Mbp and three TonB homologues (1). Therefore, according to the definition of Blanvillain *et al.*, (3) where a ratio of >5 indicates over-representation, the genome of *T. forsythia* would be considered to be over-represented for TBDRs with a TBDR/Mbp ratio of at least 17.6. This compares with genomes of other members of the *Bacteroidetes* group where TBDRs are also over-represented (e.g *B. fragilis* and *B. thetaiotaomicron* have ratios of 17.7 and 19.1, respectively) (3). The overrepresentation in *B. fragilis* and *Bacteroides thetaiotaomicron* is

considered an evolutionary adaptation to the gut environment in which they reside, with these TBDRs probably allowing them to acquire and sense a large range of host and dietary carbohydrate molecules (32,44,45,47). These putative TBDRs are also often accompanied in their genome by associated ECF-sigma and anti-sigma factors (19,32,44,45,47), and a preliminary survey of the genome of *T. forsythia* reveals that 12 of the TBDRs have partner ECF sigma and anti-sigma factors directly adjacent to them on the chromosome. These data indicate that the identified NanO TBDR, that seems to be specific for sialic acid, is part of a much larger repertoire of TBDRs that may well play a similar role for *T. forsythia* in the oral environment where it is also exposed to a range of dietary sugars as they do for gut *Bacteroides*.

In contrast to NanO, NanU has homology to other members of the SusD family, that are all predicted to be involved in nutrient utilisation of a range of carbohydrates, often oligomeric (24). It possesses a Type II Signal Recognition particle signal (18) and has predicted structural similarity to the SusD protein from *B. thetaiotaomicron* (Stafford, G.P. unpublished data). There are a number of homologues of this gene in the *T. forsythia* genome (1), again indicating that like other *Bacteroides* species this may be an important mechanism for nutrient uptake. Notably the genomes of several members of the *Bacteroidetes* also seem to possess homologues of this transport system including *B. fragilis* (NCTC 9343; *BF1719* and *1720)* and *Parabacteroides diastonis* (ATCC 8503: *BD_2944 and 2945)* both which are adjacent to sialic acid catabolic genes (Figure 1). This indicates that this type of sialic acid uptake system is present in a range of species and may therefore be important not only in the oral cavity but also in the gut. In addition, several other *Bacteroides* species (e.g. *B.* thetaiotaomicron) seem to possess NanOU homologues that are associated with fucosidase genes indicating that this family of transporter may also be involved in fucose uptake.

Clearly many questions remain regarding the mechanism of sialic acid transport via the NanOU system such as the putative protein interactions between NanO and NanU, the binding of sialic acid to NanU and the role of TonB in energising the process, which are all currently under investigation in our groups.

Sialidases appear to be multifunctional virulence factors for *T. forsythia* and other periodontal pathogens

The ability of periodontal pathogens to utilise sialic acid as a growth substrate is particularly pertinent given the range of sialylated glycoproteins present both in oral secretions (e.g. mucins) and on the surface of epithelial cells *(e.g.* fibronectin, integrins, Toll-like receptors). However, this sialic acid is not freely available and bacteria often employ secreted or membrane-bound sialidase enzymes to capture this sialic acid (Corfield, 1992). In common with many human dwelling organisms *T. forsythia* and several other periodontal pathogens such as *P. gingivalis* possess sialidase activity (Moncla, Braham, Hillier 1990) and many of the sequenced stains contain predicted *nanH* genes (Figure 1).

The *T. forsythia* NanH protein is most closely related to the sialidase of *B. fragilis* (65% identity) containing typical sialidase motifs (Roggentin *et al.*, 1993), (Thompson), in

addition to a typical putative secretion signal sequence. Recent work has shown that the *T. forsythia* NanH is able to cleave a2,3 and a2,6 sialyl bonds from both the model sugar siallyllactose (Thompson), the glycoprotein fetuin (Roy- Submitted) and sialic acid lectin binding epitopes from the surface of Ginigival epithelial cells (Honma paper), all suggesting *T. forsythia* can recover sialic acid from host-glycoproteins and that it is a significant virulence factor. In support of this we now have evidence that fetuin, mucin (less efficiently), and saliva support growth of *T. forsythia* in a sialic acid dependent manner- i.e. growth is inhibited by sialidase inhibitors and by loss of the *nanH* gene by mutation (Roy *et al.*, in submission). The idea that sialidases may be important *in vivo* for periodontal bacteria is not unexpected given that a *nanH* mutant of the related organism *B. fragilis* displayed reduced colonisation ability in rats (Godoy *et al.*, 1993) and that the periodontal pocket in which periodontal pathogens reside contains an abundance of sialylated glycoproteins (Pollanen *et al.*, 2003). Notably, all *Bacteroidetes* members that contain a nan-operon linked *nanH* gene also contain a putative 9-*O*-acetylesterase enzyme for the removal of the acetyl group at position 9 of sialic acid which is known to inhibit sialidase enzymes ref also (Figure 1). Presumably this implies that 9-O-acetylated sialic acid, which makes up a large proportion of the sialic acid in the human body e.g. 80% of the sialic acid content of mucins is o-acetylated (Varki and Diaz, 1983), is an important source of sialic acid for these organisms. This is not completely surprising since the enteric human bacterium *E. coli* has an 9-O-acetylesterase (NanS) which has specificity towards 9-*O*-acetyl sialic acid as a growth substrate (Steenbergen *et al.*, 2009). In contrast *P. gingivalis* which possesses a true sialidase (PG0352) and two O-sialoglycoproteases (PG0778 & PG1724), but no metabolic genes, does not, but may be able to cleave these linkages via the sialoglycoproteases (Fletcher).

Bacterial sialidase enzymes also play an important role in adherence to both human cells and solid surfaces (Corfield, 1992). For example, the lectin-like *Streptococcus pneumoniae* sialidase, NanA, is important for adherence to endothelial cells (Uchiyama *et al.*, 2009) while our work on *T. forsythia* highlighted a role in adhesion to oral epithelial cells (Honma) and others also showed that interaction of *T. forsythia* with blood cells can be inhibited by sialyllactose (Murakami *et al.*, 2002). The picture for *Porphyromonas gingivalis* is less clear with various sialidase and sialoglycoprotease mutants having different adhesion and invasion phenotypes (Fletcher) while pretreatment of human cells with sialidase or incubation with sialic acid reduces invasion (Hallen, Agnani). The importance of sialic acid in host interactions of periodontal pathogens though is clear and holds true for the final Red complex organism *T. denticola* whose interaction with erythrocytes is inhibited by low concentrations of sialic acid (Mixk paper).

In addition to interactions with host cells (and secretions in the oral cavity periodontal pathogens also display various interactions with other oral bacteria and in the formation of biofilm evidence body of data now suggesting a role for sialic acid and sialidases here too. For example pretreatment of *P. gingivalis* with sialidase enzyme reduces interactions with *Streptococcus sanguis* (Stinson) and we have preliminary evidence that a *nanH* mutant of *T. forsythia* aggregates less readily with the putatively sialic acid coated important bridging organism *F. polymorphum* (Bolstad *et al.*, 1996), suggesting a nutritional and physical basis

for their synergistic relationship (Sharma). Our recent work has also highlighted that *T. forsythia* sialidase is key to adhesion and biofilm formation on glycoprotein coated surfaces (Roy et al., unpub.), an observation that reflects a possible role in colonization of epithelial surfaces as was observed for the important respiratory pathogens *Pseudomonas aeruginosa* (Soong *et al.*, 2006) and *S. pneumoniae* (Parker, Soong, Planet, Brower, Ratner & Prince, 2009) King et al., 2006 (Krivan et al., 1988). The role of sialidase in these cases may be both as an adhesin itself (many have lectin like properties) but also in the exposure of underlying adhesive epitopes such as galactose, as is the case for *Bacteroides intermedius*, *Actinomyces* spp. and *Vibrio cholera* toxins (Okuda *et al.*, 1989; Gibbons *et al.*, 1990; Moustafa et al., JBC 2004). With respect to colonization by *F. nucleatum* in the oral cavity, sialidases could be involved in exposing galactose residues on host surfaces for subsequent binding by lectin-like adhesin expressed by the bacterium (Infect. Immun. 56 (1988) 1314-1319).

In addition to providing nutritional and adhesive function to organisms it is increasingly recognized that sialidases play roles in modulation of the immune responses and immune evasion. For example, *S. pneumoniae* sialidase initiates the extensive deglycosylation of secretory component and IgA1 (Molecular Microbiology (2006) (3), 961–974) as well as serum glycoproteins that results in reduced complement deposition and subsequent inhibition of killing by neutrophils (IAI 78:2108). Recent studies have also reported that full activation of Toll-like receptors in macrophage and endothelial cells that are key to the innate immune response following exposure to infectious agent molecules such as LPS, Teichoic acid and flagella is dependent on the function of host sialidases but that this can also be modified by bacterially derived sialidases with specificity for alpha 2,3 linkages (Amith, Glycoconj. J. 2009, 26:1197; Amith, Cell Signal 2010, 22:314; Stomatos et al., J. Leukoc. Biol. 2010 88: 1227). These data also suggest that the alpha 2,3 and 2,6 specific sialidases of periodontal bacteria may also contribute to the pathologic effects observed in host epithelial layers.

The potential for surface coating and host mimicry

Whether sialic acid is *de novo* synthesized or obtained exogenously by the action of sialidases on host and/or cohabiting bacterial glyconjugates, many pathogens are able to decorate their surface molecules (LPS and capsular polysaccharide) with sialic acids and its derivatives in order to mimic host cell surfaces, e.g. *H. influenzae, N. meningitidis*, *E. coli* K1 and *C. jejuni* (refs). This ‘ molecular mimicry’ helps in the avoidance of host immune attack (Vimr and Lichtensteiger, 2002, Severi). Moreover, surface sialic acid expression is also believed to hinder the reach of complement dependent membrane attack complex (MAC on the bacterial membrane. With regard to bactera of the oral cavity, the *F. polymorphum* genome possesses (www.oralgen.org) putative genes *(FNP_1104-FNP_1109)* which encodes *neuC, neuA, neuB*, N-acetylneuraminate synthase, a polysaccharide biosynthesis export protein and lipooligosaccharide sialyltransferase (lst respectively (http://www.oralgen.lanl.gov/) that might be involved in sialylation of LPS. It remains to be seen whether LPS sialylation does occur in *F. polymorphum* and if it plays any role in immune mimicry.

However, to date, no other periodontal bacteria with gene homologues indicating LPS sialylation have been reported/documented.

In addition to LPS and capsule sialylation it is becoming clear that many bacteria are capable of glycosylation of surface proteins such as flagella (refs) and outer membrane proteins (bfrag refs). One such example is the S-layer of *T. forsythia* which is known to be heavily glycosylated. However, the nature of this glycosylation is unclear and the only hints as to its composition come from a mutation in a putative N-acetyl-D-mannosaminuronic acid dehydrogenase gene (wecC that results in a defect in protein glycosylation (Honma ref) and in the presence of several potential fucosylation sites of the type identified in *B. fragilis* by xxxxx (ref). While there is no evidence of sialylation of the S-layer thus far it is tempting to speculate that this might be the case, especially given that the related organism *P. gingivalis* (which also lacks sialylation pathways) contains sialic acid attached to the major surface gingipain, Rgp (Rangarajan). One possible mechanism for this could be trans-sialylation via sialidase enzymes as has been observed in eukaryotes in recent years.

Interestingly, the molecular mimicry reaches near perfection in another human commensal/pathogen, the Group B *Streptococci* (GBS). GBS express capsular polysaccharide that displays the glycan structure Siaα-2-3Galβ1-4GlcNac, found in human glycoproteins recognized by Sia-recognizing immunoglobulin superfamily lectin (Siglecs) receptors on immune cells, including neutrophils. Siglec receptors, due to the presence of cytoplasmic tyrosine based inhibitory motif (ITIM are believed to dampen the inflammatory responses following host sialoglycan binding. Thus, molecular mimicy by GBS has been postulated to impair neutrophil defense functions by coopting Siglecs. The interaction with Siglecs can also can lead to other consequences, such as in the case of *C. jejnui* where reconition of sialic acid containing antigens contributes to autoimmune disorders such as Guillan-Barre syndrome (Carlin *et al.*, 2007;Ilg *et al.*, 2010).

Whether any or all of these phenomena are present in periodontal pathogens is not clear at present but there is clearly much scope for this to be the case, a fact that would add to the complexity of interactions between these bacteria and the host immune system.

A Model for the role of sialic acid in periodontitis

Overall there is a growing body of evidence and opinion that sialic acid plays a key role in the life and consequences of periodontal pathogen colonisation (Figure 3). There seems little doubt that at least for some periodontal organisms sialic acid can act as a growth and adhesion factor for colonisation both of host surfaces but also probably in interactions with other oral dwelling bacteria within biofilm. The production of sialidase enzymes by a range of periodontal pathogens and the obvious potential therefore for modulation of the immune response means there is much to investigate and much scope for the design of new treatment regimen as well as an increased understanding of the microbial community in the oral cavity.

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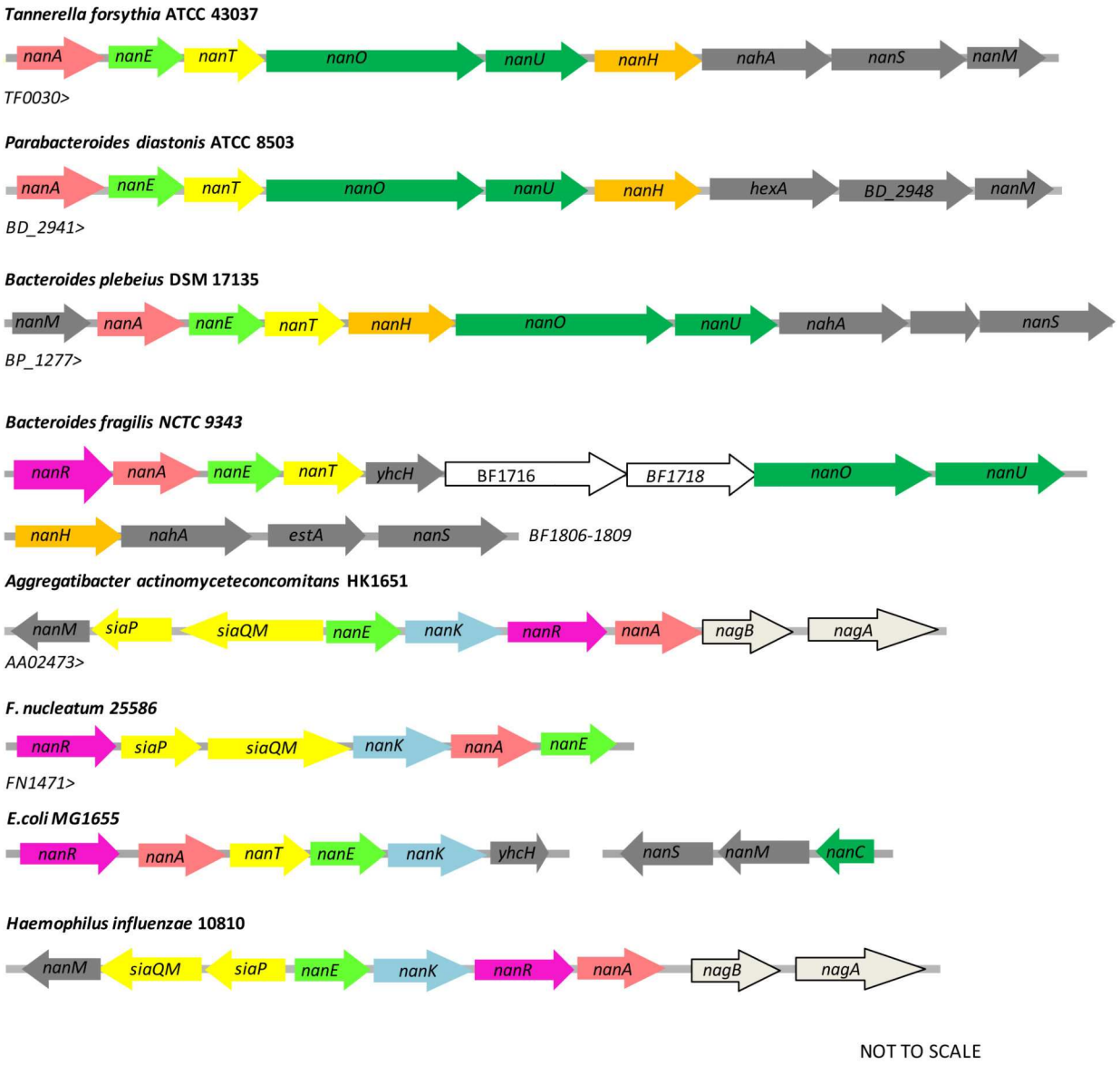

Figure 1. Sialic acid catabolism and transport clusters from a range of bacteria

Predicted and confirmed sialic acid gene clusters from the genome sequences of the organisms shown are illustrated using standard *nan* gene descriptors. Key: Catabolic genes: *nanA*-neuraminate lyase(red), nanE-N-acetylmannosamine-6P epimerase (lime green), *nanK*-ManNAc kinase (turquoise) ; Inner membrane transporters (yellow) : *nanT*- Major facilitator Superfamily permease; *siaPQM*- Neu5Ac TRAP (tripartite ATP-independent periplasmic) transporter; Outer membrane transporter (mid green) : *nanOU*- TonB dependent sialic acid transport system, *nanC*-sialic acid specific porin; Accesory genes (Grey) : *nanS*- sialic acid 9-O-acetylesterase; *nahA/hexA*-beta hexosaminidase; *nanM*- sialic acid mutarotase; *yhcH*- putative Glycolyl sialic acid processing enzyme; *estA*- sialyl transferase. The first gene in each cluster is noted for each species except the well established *E. coli* and *H. influenzae.*

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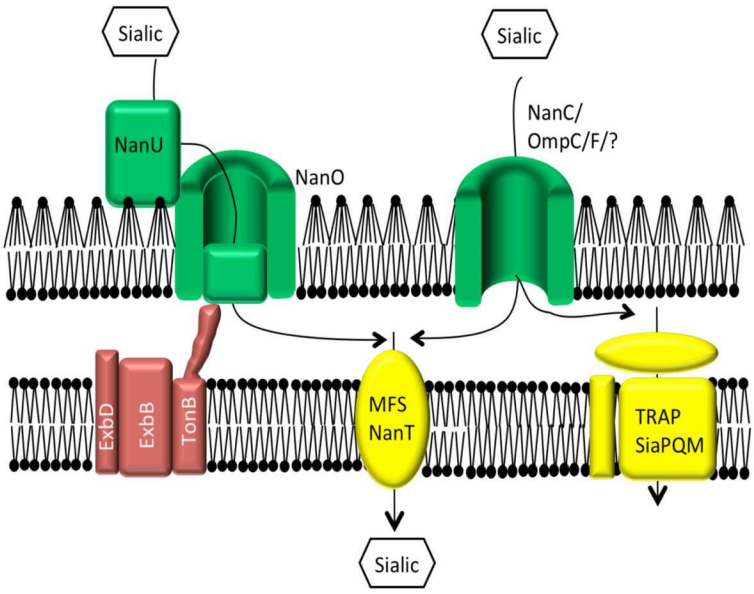

Figure 2. Summary of sialic acid uptake systems present in periodontal bacteria

Sialic acid enters either via either a TonB dependent NanOU type system before entry into the cytoplasm via a NanT MFS permease protein. In organisms that lack a NanOU system it is likely that they employ a NanC type or general porin like OmpC or OmpF that is used in *E. coli*. This might then feed either to a NanT permease or a SiaPQM TRAP type permease as is the case for *H. influenzae*.

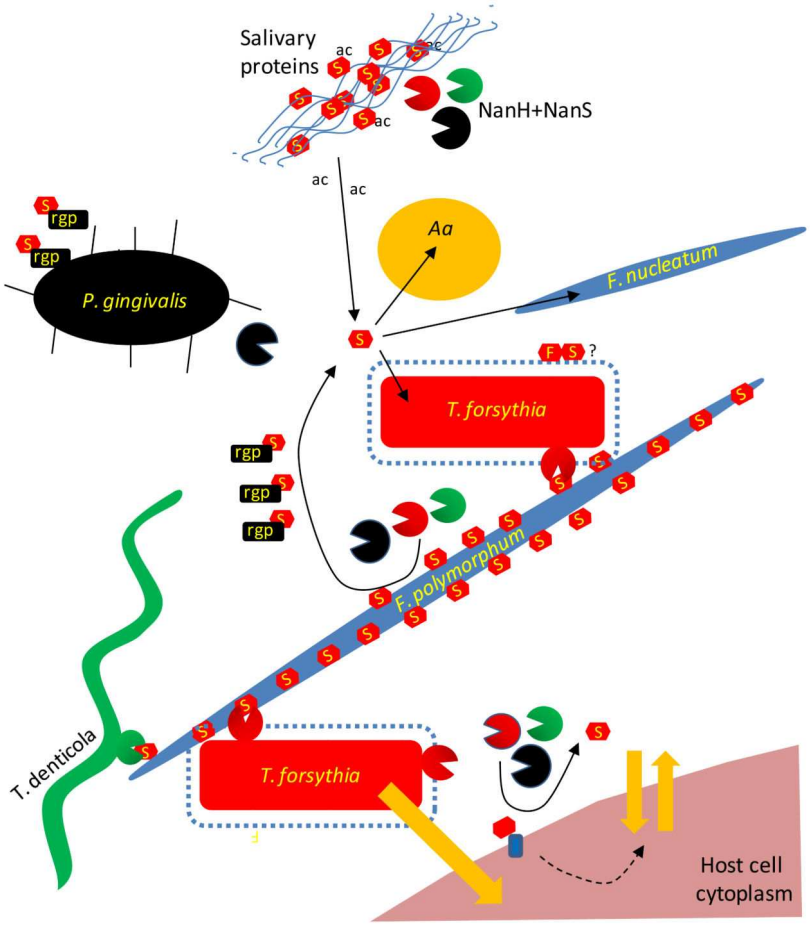

Figure 3. Model of sialic acid dependent interactions of periodontal pathogens

All of the pathogens shown except for the *Fusobacteium spp.* Produce cell-anchored or secreted sialidase enzymes (colour coded Pacman for parent strain) that potentially release sialic acid from both bacterial, e.g. LPS of *F. polymorphum* or arginine gingipain (rgp) of *P. gingivalis*, and host sources, e.g. cell surface or salivary glycoproteins. The consequences of this removal of sialic acid may also be inter-bacterial interaction or activation of host cell signaling cascades (orange arrows) such as TLR pathways or cytokine release. Key: Ac- acetyl group, ‘ s’ in a hexagon- sialic acid residue, Aa*Aggregatibacter actnimomyceteconcomitans*, rgp- arginine gingipain.