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TURNING *IN SILICO* PHAGE INTO *IN VIVO* PHAGE: THE CRASSPHAGE STORY

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SUMMARY

CrAss-like phages were originally identified as highly abundant and ubiquitous members of the gut microbiome from studies involving metagenomic sequencing and cross-assembly of reads from multiple individuals (**crAss** - **Cr**oss **Ass**embly). More recently, it has been established that crAss-like phages infect bacteria of the phylum Bacteroidetes. One of the most interesting facets to crAss-like phages is their ability to persist at high numbers over extended periods in the laboratory and in the gut without significantly impacting the abundance of their bacterial hosts. Here we recount what is known about crAss-like phages so far, from their *in silico* discovery in 2014 and the subsequent identification of unique genomic features, to the first isolation of crAss001 and the elucidation of various biological characteristics resulting from the study of phage-host pairs *in vivo*. A large amount of information has been gleaned in a relatively short period of time but it is clear that crAss-like phage research is still in its infancy. Future research lies in further *in vivo* work with phage-host pairs, coupled with the isolation of further crAss-like phage from more divergent groups.

INTRODUCTION

CrAss-like phages are an intriguing member of the human gut microbiome. They are both extremely prolific and widespread, and account for over 86% of gut viral genomes. (*Yutin* et al., 2021) They have been identified in faecal samples from all over the globe, and are found in all age groups from infants to the elderly (*Edwards* et al., 2019). While crAssphages are rarely a component of the new-born microbiome, they become increasingly prevalent within the first year of life. It has been suggested that vertical transmission leads to this initial colonisation (*McCann* et al., 2018; *Siranosian* et al., 2020). They have also been shown to be transferred and to stably engraft in recipients of faecal microbiota transplants (*Draper* et al., 2018). CrAss-like phages have been identified in both remote and traditional hunter-gatherer populations, albeit at a reduced prevalence to that of industrialised populations (*Edwards* et al., 2019; *Honap* et al., 2020). CrAss-like phages have also been identified in sequences recovered from old and new world primates, suggesting an ancient association of crAss-like phages with these primates (*Edwards* et al., 2019). CrAss-like phages are generally viewed as benign viruses of the gut. Correlations between crAss-like phages and a number of different health and disease factors have been examined, but no significant associations have been identified (*Honap* et

al., 2020). At the same time, one crAss-like phage, termed IAS virus, was identified in stool samples from HIV-1 patients with unexplained diarrhoea. While no correlations between IAS virus and diarrhoea were established, it was significantly associated with advanced stages of HIV-1 disease (*Oude Munnink* et al., 2014).

IN SILICO DISCOVERY

The founding member of the CrAss-like phages was originally identified *in silico* in 2014 (*Dutilh* et al., 2014). Despite being both highly abundant and ubiquitous, its divergence from other bacteriophage sequences in viral databases allowed it to remain a component of the 'viral dark matter' for decades. Identification of the ~97 Kb dsDNA genome of this prototypical crAssphage (p-crAssphage) came from the re-analysis of publicly available viral metagenomes using cross-assembly software (crAss), from which crAssphage derives its name. A reference independent method, crAss cross-assembles all reads from input samples and counts the number of shared contigs (*Dutilh* et al., 2012). At the time of assembly, fewer than 20 of the 80 predicted ORF's could be assigned a function. While the phage had never been grown in the laboratory, CRISPR spacers and co-occurrence analysis predicted that *Bacteroides* was the likely host of this phage.

Subsequent studies identified variations to the p-crAssphage genome (*Manrique* et al., 2016; *Liang* et al., 2016). This was expanded on by Yutin and colleagues (*Yutin* et al*.,* 2018) in which the use of sensitive protein analysis methods revealed several hundreds of putative members of a wider crAss-like family, found not only in the human gut but also in diverse environments such as groundwater, marine sediments and hypersaline brine. Annotation of a number of putative tail proteins with homologs to phage P22 led to the prediction that crAss-like phages encode for short tails, typical of that of *Podoviridae.* This was later supported by transmission electron microscopy (TEM) images of a faecal filtrate rich in crAss-like phages that was dominated by podoviridae (*Guerin* et al., 2018).

CURRENT CLASSIFICATIONS AND GENERAL GENOMIC FEATURES

Hundreds of additional CrAss-like phage genomes (each assembled from sequences derived from a single individual) have been further classified into four subfamilies (Alphacrassvirinae, Betacrassvirinae, Gammacrassvirinae and Deltacrassvirinae) and ten genera (I - X), based on the percentage of protein coding genes shared between phages. CrAss-like phages sharing > 40% of protein coding genes were classified at the genus level, while CrAss-like phages sharing 20-40% were classified at the subfamily level. (*Guerin* et al., 2018) Recent analysis by Yutin and colleagues (*Yutin* et al., 2021) identified almost 600 crAss-like genomes and expanded classification to include two more groups, epsilon and zeta. It has been proposed that crAss-like phages could form an order within the class Caudoviricetes, with 6 families, comprising the existing 4 subfamilies and the additions of epsilon and zeta (*Walker* et al., 2021).

In general, crAss-like phages have a genome size of ~100 Kb, although they can range from as large as ~150 - 200

Kb in the case of groups epsilon and zeta. Their genomes are divided roughly in two modules, with opposite gene orientation. One side harbours genes responsible for replication and nucleotide metabolism while the other encodes for structural genes (*Yutin* et al., 2018; *Shkoporov* et al., 2018; *Guerin* et al., 2021). At the junction of the two opposite modules 2-3 genes encode a very large RNA polymerase (RNAP) of up to 6000 amino acids in total. The genes encoding these subunits are sometimes on opposite coding strands. These subunits are highly divergent both from each other and other known RNAP proteins. They have been confirmed to be structurally most similar to eukaryotic RNAP that are involved in RNA interference (*Drobysheva* et al., 2020). Analysis of the first crAssphage to have been grown in culture confirms that this large RNAP is a component of the virion and are almost certainly involved in transcription of early phage genes (*Shkoporov* et al., 2018; *Drobysheva* et al., 2020). Why crAss-like phage encode such abnormally large genes for these subunits is unclear.

In addition to these large RNAP genes, crAss-like phages possess a number of other unusual genomic features. Most encode a DNA polymerase from either the A or B family and switching can occur between the two, even within the same subfamily (*Yutin* et al., 2018, 2021). A large number of crAss-like phages, mainly from the beta, zeta and epsilon groups, appear to use alternative genetic codes (*Guerin* et al., 2018; *Yutin* et al., 2021). Typically, this is by the reassignment of stop codon TAG to glutamine or TGA to tryptophan and seems to mainly occur in the late phage genes. Some crAss-like phages, particularly of the zeta group, are characterised by an abnormally high density of introns and inteins *(Yutin* et al., 2021). Whether this spread of mobile genetic elements is uncontrollable due to some aspect of the phage lifestyle or is an adaptive approach used by the phage is so far unknown. Those crAss-like phages isolated to date, p-crAssphage and others used in comparative studies have lacked these features, leading to relatively simple annotation.

IN VITRO ISOLATION AND BIOLOGICAL PROPERTIES

To date, five crAss-like phages have been isolated with their hosts, ΦCrAss001, ΦCrAss002, DAC15, DAC17 and Φ14:2, which infect hosts *Bacteroides intestinalis, Bacteroides xylanisolvens, Bacteroides thetaiotaomicron* and *Cellulophaga baltica* respectively, confirming the previous predictions of *Bacteroidetes* as a host (*Shkoporov* et al., 2018; *Hryckowian* et al., 2020; *Guerin* et al., 2021; *Holmfeldt* et al., 2013). ΦCrAss001 and its closely related phages DAC15 and DAC17 all belong to the subfamily Betacrassvirinae, while ΦCrAss002 is more closely related to p-crAssphage, as it falls into the category of Alphacrassvirinae. Φ14:2 is a distantly related crAss-like phage, isolated from seawater. Our limited knowledge of the biological properties of crAss-like phages comes from the studies of these phage-host pairs.

CrAss-like phages appear to establish an equilibrium with their hosts in liquid culture, in which they can propagate stably over many subcultures, without causing lysis of their hosts. This has also been demonstrated in a mouse model, where CrAss001 can maintain total counts between approximately 10^6-10^8 pfu/mL over a period of 136 days, without causing a reduction in

B. intestinalis counts (*Shkoporov* et al., 2021). This corresponds with a longitudinal study of ten human volunteers, where crAss-like phages were able to stably persist within individuals over a period of one year (*Shkoporov* et al., 2019). The Bacteroides hosts typically encode a number of alternative capsular polysaccharides (CPS) within their genomes, some which seem to be permissive to phage infection, while others are likely to be neutral or protective (*Porter* et al., 2020). Phase variation of these CPS's appear to help to maintain the bacterial population in the presence of phages by creating a resistant subpopulation. The switch to a resistant subtype can occur even in the absence of phages (*Shkoporov* et al., 2021). DAC15 and DAC17 can infect the wild type version of *B. thetaiotaomicron,* which produces 8 different CPS's. On the wild type strain they produce hazy plaques and can be maintained in liquid culture at 10^7-10^8 pfu/mL. When infecting an engineered strain that produces only CPS3, clear plaques are formed and phage counts are maintained at a level one log higher in liquid culture (*Shkoporov* et al., 2021). Interestingly, lysis of the cell culture still does not occur, suggesting that other mechanisms, such as phase variation of outer membrane lipoproteins, may also contribute to the dynamic equilibrium between both partners (*Porter* et al., 2020).

A second mechanism, likely to be working in parallel to phase variation, is an apparent delayed release of phage progeny from infected cells. In classic one step growth curves at an MOI of 1, ΦCrAss001, DAC15 and DAC17 all generate very small burst sizes of between 2.5-8 pfu per infected cell (*Shkoporov* et al., 2018, 2021). In the case of ΦcrAss001, it has been observed that a second small burst occurs around 90 minutes after the first. Despite this, more than 50 phage particles per cell can be observed in TEM's of ΦCrAss001 spotted onto lawns of *B. intestinalis*, and at least 20 copies of ΦCrAss001 genome per copy of the *B. intestinalis* genome are produced within 90 minutes of infection. Intriguingly, this mechanism of delayed release can be overcome by increasing the MOI in *B. theta* but the same cannot be observed for *B. intestinalis* (*Shkoporov* et al., 2021).

While ΦCrAss001, DAC15 and DAC17 have all been demonstrated to behave quite similarly, ΦCrAss002 has a number of additional interesting properties. It is unable to form plaques on its host and can only form opaque spots that are barely visible when a highly concentrated phage stock is spotted onto a lawn of its sensitive bacterial host. Furthermore, propagation in liquid culture only occurs after a minimum of 3 days subculturing and it appears that it is *B. xylanisolvens* that adapts to allow phage infection. Why adaptation by the host is delayed is unknown. Phase variation is a possible contributor here too, with *B. xylanisolvens* possessing many recombination hotspots (*Guerin* et al., 2021).

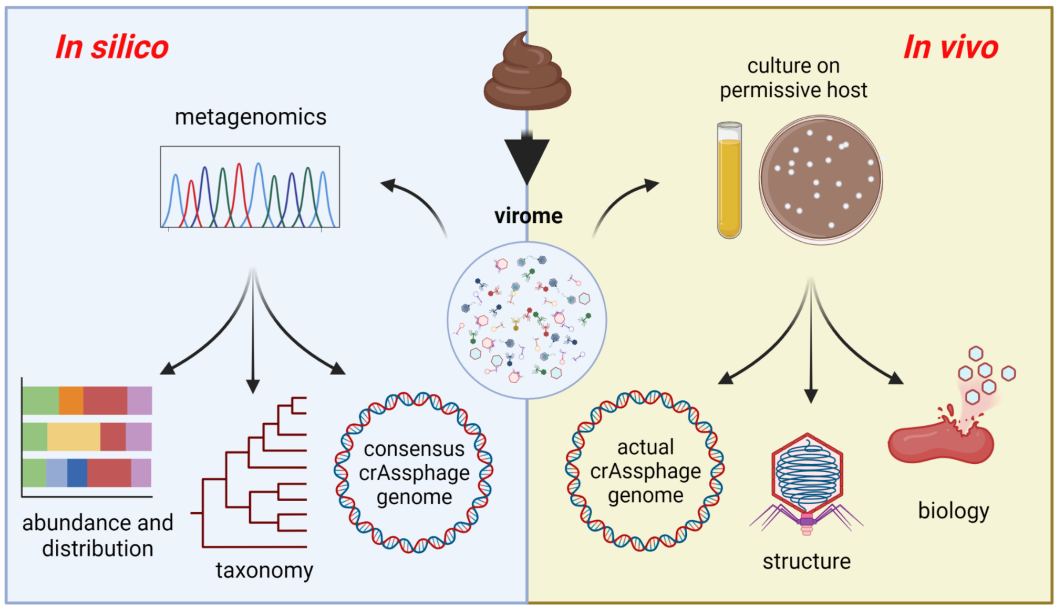

Figure 1: Both *in silico* and *in vivo* approaches can provide important information about the crAsslike phages, but a true understanding of these highly abundant viruses (especially in terms of their biology, structure, and genomic features) will depend on our ability to grow them in the laboratory. Created using BioRender.com.

ECOLOGICAL MODELS

A number of different models have been described to explain bacteriophage lifestyles and their interactions with their hosts in their environment. While often used to describe phage-host dynamics in the ocean, they can be extrapolated to illustrate interactions within the human gut. The "piggyback the winner" model has traditionally been viewed as a switch from a lytic to temperate lifestyle, in order to keep host levels at a high abundance (*Knowles* et al., 2016). However, recent work has suggested that a healthy human virome is dominated by a lytic core of crAss-like phages and *Microviridae* (*Shkoporov* et al., 2019). It is possible that the use of mechanisms such as phase variation and delayed release of phage progeny by crAss-like phages correspond with alternative mechanisms of benign infection that align with the piggyback the winner model. These less aggressive means of replication may allow the bacterial population to persist to a high abundance, therefore enabling crAss-like phage persistence.

It is also possible that other models are at play, such as "Royal Family" dynamics. This is where the "kill the winner" model operates at the strain level, such that any fluctuations in phage or host populations would then go undetected (*Breitbart* et al., 2018). Recently, enrichment of nonsynonymous variants has been described in tail protein encoding genes of crAss-like phages (*Brown* et al., 2021; *Siranosian* et al., 2020). As tail proteins are often important mediators in bacterial host recognition, it is possible that crAss-like phages use these tail protein variants to switch hosts at the strain level, in line with Royal family dynamics. Possibly,

crAss-like phages use a number of different mechanisms from different models in combination, to enable them to persist stably, without affecting host abundance.

CONCLUSIONS

In just eight years, CrAss-like phages have gone from a viral unknown, to being recognised as the most abundant phages of the human gut, with sequences isolated all over the world. *In silico* analysis have revealed an array of interesting genomic features but many of their biological purposes remain unclear. Isolation of the first few phage-host pairs has allowed the elucidation of some of the biological characteristics of these phages (Figure 1). However, three of the five isolated crAss-like phages come from the Betacrassvirinae group, which have been shown to make up just < 1.5% of the crAss-like phages dominating the human gut (*Yutin* et al., 2021). CrAss-like phages from Alphacrassvirinae and Gammacrassvirinae groups dominate the gut virome, combined they make up just under half of all crAss-like phages. ΦCrAss002 is the only isolated crAssphage from the Alphacrassvirinae and it has shown to exhibit a number of biological mechanisms that are different from those of the Betacrassvirinae phages. It is likely that crAss-like phages employ a number of different mechanisms that allow them to persist so stably within the human gut. Isolation of members from other subfamilies will be required in order to garner further insights into these mechanisms. This will not be an easy task given that the hosts of these phages are Bacteroidetes, strict anaerobes that are difficult to isolate in their own right. The use of alternative genetic codes and the presence of introns and inteins in some of the crAss-like phage groups also add to the difficulty of correct annotation. It is vital that we continue to culture new members of this extensive and newly established phage order - the *Crassvirales*. Only by growing these phages in the laboratory will we begin to truly understand their role in the gut microbiota. An additional benefit will be structural studies that will allow us to assign functions to the many unannotated genes within crAssphage genomes. As the most abundant phages of the human gut, it is befitting that they should also become the most studied.

LITERATURE

Breitbart, M., Bonnain, C., Malki, K., and Sawaya, N.A.: Phage puppet masters of the marine microbial realm. Nat. Microbiol. 3, 754-766 (2018).

Brown, B.P., Chopera, D., Havyarimana, E., Wendoh, J., Jaumdally, S., Nyangahu, D.D., Gray, C.M., Martin, D.P., Varsani, A., and Jaspan, H.B.: crAssphage genomes identified in fecal samples of an adult and infants with evidence of positive genomic selective pressure within tail protein genes. Virus Res. 292, 198219 (2021).

Draper, L.A., Ryan, F.J., Smith, M.K., Jalanka, J., Mattila, E., Arkkila, P.A., Ross, R.P., Satokari, R., and Hill, C.: Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. Microbiome 6, 220 (2018).

Drobysheva, A.V., Panafidina, S.A., Kolesnik, M.V., Klimuk, E.I., Minakhin, L., Yakunina, M.V., Borukhov, S., Nilsson, E., Holmfeldt, K., Yutin, N., Makarova, K.S., Koonin, E.V., Severinov, K.V., Leiman, P.G., and Sokolova, M.L.: Structure and function of

virion RNA polymerase of a crAss-like phage. Nature 589, 306-309 (2020).

Dutilh, B.E., Cassman, N., McNair, K., Sanchez, S.E., Silva, G.G.Z., Boling, L., Barr, J.J., Speth, D.R., Seguritan, V., Aziz, R.K., Felts, B., Dinsdale, E.A., Mokili, J.L., and Edwards, R.A.: A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. Nat. Commun. 5, 4498 (2014).

Dutilh, B.E., Schmieder, R., Nulton, J., Felts, B., Salamon, P., Edwards, R.A., and Mokili, J.L.: Reference-independent comparative metagenomics using cross-assembly: crAss. Bioinformatics 28, 3225-3231 (2012).

Edwards, R.A., Vega, A.A., Norman, H.M., Ohaeri, M., Levi, K., Dinsdale, E.A., Cinek, O., Aziz, R.K., McNair, K., Barr, J.J., Bibby, K., Brouns, S.J.J., Cazares, A., de Jonge, P.A., Desnues, C., Díaz Muñoz, S.L., Fineran, P.C., Kurilshikov, A., Lavigne, R., Mazankova, K., McCarthy, D.T., Nobrega, F.L., Reyes Muñoz, A., Tapia, G., Trefault, N., Tyakht, A.V., Vinuesa, P., Wagemans, J., Zhernakova, A., Aarestrup, F.M., Ahmadov, G., Alassaf, A., Anton, J., Asangba, A., Billings, E.K., Cantu, V.A., Carlton, J.M., Cazares, D., Cho, G.S., Condeff, T., Cortés, P., Cranfield, M., Cuevas, D.A., De la Iglesia, R., Decewicz, P., Doane, M.P., Dominy, N.J., Dziewit, L., Elwasila, B.M., Eren, A.M., Franz, C., Fu, J., Garcia-Aljaro, C., Ghedin, E., Gulino, K.M., Haggerty, J.M., Head, S.R., Hendriksen, R.S., Hill, C., Hyöty, H., Ilina, E.N., Irwin, M.T., Jeffries, T.C., Jofre, J., Junge, R.E., Kelley, S.T., Khan Mirzaei, M.K., Kowalewski, M., Kumaresan, D., Leigh, S.R., Lipson, D., Lisitsyna, E.S., Llagostera, M., Maritz, J.M., Marr, L.K., McCann, A., Molshanski-Mor, S., Monteiro, S., Moreira-Grez, B., Morris, M., Mugisha, L., Muniesa, M., Neve, H., Nguyen, N.P., Nigro, O.D., Nilsson, A.S., O'Connell, T., Odeh, R., Oliver, A., Piuri, M., Prussin li, A.J, Qimron, U., Quan, Z.X., Rainetova, P., Ramírez-Rojas, A., Raya, R., Reasor, K., O Rice, G.A., Rossi, A., Santos, R., Shimashita, J., Stachler, E.N., Stene, L.C., Strain, R., Stumpf, R., Torres, P.J., Twaddle, A., Ugochi Ibekwe, M.U., Villagra, N., Wandro, S., White, B., Whiteley, A., Whiteson, K.L., Wijmenga, C., Zambrano, M.M., Zschach, H., Dutilh, B.E.: Global phylogeography and ancient evolution of the widespread human gut virus crAssphage. Nat. Microbiol. 4, 1727-1736 (2019).

Guerin, E., Shkoporov, A.N., Stockdale, S.R., Comas, J.C., Khokhlova, E.V., Clooney, A.G., Daly, K.M., Draper, L.A., Stephens, N., Scholz, D., Ross, R.P., and Hill, C.: Isolation and characterisation of ΦcrAss002, a crAss-like phage from the human gut that infects Bacteroides xylanisolvens. Microbiome 9, 89 (2021).

Guerin, E., Shkoporov, A., Stockdale, S.R., Clooney, A.G., Ryan, F.J., Sutton, T.D.S., Draper, L.A., Gonzalez-Tortuero, E., Ross, R.P., and Hill, C.: Biology and Taxonomy of crAss-like Bacteriophages, the Most Abundant Virus in the Human Gut. Cell Host Microbe 24, 653-664 (2018)

Holmfeldt, K., Solonenko, N., Shah, M., Corrier, K., Riemann, L., VerBerkmoes, and N.C., Sullivan, M.B.; Twelve previously unknown phage genera are ubiquitous in global oceans. Proc. Natl. Acad. Sci. USA 110, 12798-12803 (2013).

Honap, T.P., Sankaranarayanan, K., Schnorr, S.L., Ozga, A.T., Warinner, C., and Lewis Jr, C.M.: Biogeographic study of human gut-associated crAssphage suggests impacts from industrialization and recent expansion. PLoS One 15, e0226930 (2020).

Hryckowian, A.J., Merrill, B.D., Porter, N.T., Van Treuren, W., Nelson, E.J., Garlena, R.A., Russell, D.A., Martens, E.C., Sonnenburg, J.L.: Bacteroides thetaiotaomicron-Infecting Bacteriophage Isolates Inform Sequence-Based Host Range Predictions. Cell Host Microbe 28, 371-379 (2020).

Knowles, B., Silveira, C.B., Bailey, B.A., Barott, K., Cantu, V.A., Cobián-Güemes, A.G., Coutinho, F.H., Dinsdale, E.A., Felts, B., Furby, K.A., George, E.E., Green, K.T., Gregoracci, G.B., Haas, A.F., Haggerty, J.M., Hester, E.R., Hisakawa, N., Kelly,

L.W., Lim, Y.W., Little, M., Luque, A., McDole-Somera, T., McNair, K., de Oliveira, L.S., Quistad, S.D., Robinett, N.L., Sala, E.S., Salamon, P., Sanchez, S., Sandin, S., Silva, G.G.Z., Smith, J., Sullivan, C., Thompson, C., Vermeij, M.J.A., Youle, M., Young, C., Zgliczynski, B., Brainard, R.A., Edwards, R., Nulton, J., Thompson, F., and Rohwer, F.: Lytic to temperate switching of viral communities. Nature 531, 466-470 (2016).

Liang, Y.Y., Zhang, W., Tong, Y.G., and Chen, S.P.: crAssphage is not associated with diarrhoea and has high genetic diversity. Epidemiol. Infect. 144, 3549-3553 (2016).

Manrique, P., Bolduc, B., Walk, S.T., van der Oost, J., de Vos, W.M., and Young, M.J.: Healthy human gut phageome. Proc. Natl. Acad. Sci. USA 113, 10400-10405 (2016).

McCann, A., Ryan, F.J., Stockdale, S.R., Dalmasso, M., Blake, T., Ryan, C.A., Stanton, C., Mills, S., Ross, P.R., and Hill, C.: Viromes of one year old infants reveal the impact of birth mode on microbiome diversity. PeerJ. 6, e4694 (2018).

Oude Munnink, B.B., Canuti, M., Deijs, M., de Vries, M., Jebbink, M.F., Rebers, S., Molenkamp, R., van Hemert, F.J., Chung, K., Cotten, M., Snijders, F., Sol, C.JA., and van der Hoek, L.: Unexplained diarrhoea in HIV-1 infected individuals. BMC Infect. Dis. 14, 22 (2014).

Porter, N.T., Hryckowian, A.J., Merrill, B.D., Fuentes, J.J., Gardner, J.O., Glowacki, R.W.P., Singh, S., Crawford, R.D., Snitkin, E.S., Sonnenburg, J.L., and Martens, E.C.: Phase-variable capsular polysaccharides and lipoproteins modify bacteriophage susceptibility in Bacteroides thetaiotaomicron. Nat. Microbiol. 5, 1170-1181 (2020).

Shkoporov, A.N., Clooney, A.G., Sutton, T.D.S., Ryan, F.J., Daly, K.M., Nolan, J.A., McDonnell, S.A., Khokhlova, E.V., Draper, L.A., Forde, A., Guerin, E., Velayudhan, V., Ross, R.P., and Hill, C.: The Human Gut Virome Is Highly Diverse, Stable, and Individual Specific. Cell Host Microbe 26, 527-541 (2019).

Shkoporov, A.N., Khokhlova, E.V., Fitzgerald, C.B., Stockdale, S.R., Draper, L.A., Ross, R.P., and Hill, C.: ΦCrAss001 represents the most abundant bacteriophage family in the human gut and infects Bacteroides intestinalis. Nat. Commun. 9, 4781 (2018).

Shkoporov, A.N., Khokhlova, E.V., Stephens, N., Hueston, C., Seymour, S., Hryckowian, A.J., Scholz, D., Ross, R.P, and Hill, C.: Long-term persistence of crAss-like phage crAss001 is associated with phase variation in Bacteroides intestinalis. BMC Biol. 19, 163 (2021).

Siranosian, B.A., Tamburini, F.B., Sherlock, G., and Bhatt, A.S.: Acquisition, transmission and strain diversity of human gut-colonizing crAss-like phages. Nat. Commun. 11, 280 (2020)

Walker, P.J., Siddell, S.G., Lefkowitz, E.J., Mushegian, A.R., Adriaenssens, E.M., Alfenas-Zerbini, P., Davison, A.J., Dempsey, D.M., Dutilh, B.E., García, M.L., Harrach, B., Harrison, R.L., Hendrickson, R.C., Junglen, S., Knowles, N.J., Krupovic, M., Kuhn, J.H., Lambert, A.J, Łobocka, M., Nibert, M.L., Oksanen, H.M., Orton, R.L., Robertson, D.L., Rubino, L., Sabanadzovic, S., Simmonds, P., Smith, D.B., Suzuki, N., Van Dooerslaer, K., Vandamme, A., Varsani, A. and Zerbini, F.M.: Changes to virus taxonomy and to the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2021). Arch. Virol. 166, 2633-2648 (2021).

Yutin, N., Benler, S., Shmakov, S.A., Wolf, Y.I., Tolstoy, I., Rayko, M., Antipov, D., Pevzner, P.A., and Koonin, E.V.: Analysis of metagenome-assembled viral genomes from the human gut reveals diverse putative CrAss-like phages with unique genomic features. Nat. Commun. 12, 1044 (2021).

Yutin, N., Makarova, K.S., Gussow, A.B., Krupovic, M., Segall, A., Edwards, R.A., and Koonin, E.V.: Discovery of an expansive bacteriophage family that includes the most abundant viruses from the human gut. Nat. Microbiol. 3, 38-46 (2018).