Review

Structural, functional and molecular basis of cyanophage-cyanobacterial interactions and its significance

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The cyanophages are double-stranded deoxyribonucleic acid (ds-DNA) viruses, infecting cyanobacteria which are the first oxygenic photosynthesizers and significant nitrogen fixers of the biosphere. The evolutionary findings of the cyanophages do not truly reflect their actual time of origin. They show extreme diversification in morphology, habitat, host range and molecular attributes. They infect and establish an association with the cyanobacterial cells through one of the two modes of multiplication, that is, lytic or lysogenic type which might be dependent upon the environmental signals, such as nutritional status, the presence of any pathogenic condition and the relative concentration of either the control of repressor’s operator/clear 1 (Cro/CI) protein that embody the bistable genetic switched regulators that are responsible to a large extent for the functioning of any of the above cycle. Various environmental factors that affect the stability and sustenance of the phages were meticulously reviewed. Genetic exchange and gene shuffling might be responsible for the enormous structural, functional, ecological and molecular diversity of the cyanophages. The cyanophages maintain the ecological equilibrium by keeping the nutrient cycling and microbial diversity at an appropriate level. Therefore, this review covers all the known, lesser acknowledged facets related to phage biology and clearly emphasizes the need for advanced molecular studies on the cyanophage-cyanobacterial association.

Key words: Cyanophage, cyanobacteria, cyanophages-cyanobacterial interaction.

INTRODUCTION

Viruses are the most common life forms in the aquatic environment. They are known to be an abundant and dynamic component in the aquatic microbial communities that can regulate the biomass production and species composition of varied microorganisms and other life forms like bacteria and phytoplankton. Due to their enormous abundance, ubiquity and significant impact on both biogeochemical and ecological cycles through the infection and lysis of bacterial and phytoplankton communities (Fuhrman, 1999; Wilhelm and Suttle, 1999; Hewson and Fuhrman, 2008; Sandaa, 2008; Thurber, 2009) they have been considered as a key component of the aquatic ecosystem. They are even known to mediate gene transfer between microorganisms in aquatic ecosystems (Bergh et al., 1989; Suttle et al., 1990; Thingstad et al., 1993; Fuhrman, 1999). First time isolation of a virus that infected filamentous cyanobacteria was reported by Safferman and Morris (1963). These viral agents have been previously named as phycoviruses (Schneider et al., 1964), algophages and blue-green algal viruses (Safferman and Morris, 1964; Luftig and Haselkorn, 1967). The term ‘Cyanophage’ seems to be the most appropriate because of the close resemblance with the term ‘bacteriophage’ and there are many reports showing analogy of the cyanobacteria to the bacteria (Stanier and Van-Niel, 1962). The blue-green algae appeared in middle precambrian rock or even earlier and are considered as one of the most ancient organisms on the earth (Schopf, 1970). Before the advent of the cyanophages, the photoautotrophic metabolism
was studied in the tissues of higher plants under viral infection but the results were far from satisfaction and inconsistent because the plant system which is consisted of different tissue systems, were difficult to isolate in tissue cultures and did not allow for homogeneous infection.

Due to the resemblance between cyanophages and the bacteriophages as well as the presence of common type of photosynthetic activity in blue-green algae and higher plants provide an ideal and excellent model system to study the photosynthetic activity under viral infection. Lots of works have been done related to the isolation and characterization of cyanophages system and also their potentiality to control cyanobacterial bloom in aquatic system (Padan and Shilo, 1973; Stewart and Daft, 1977; Sherman and Brown, 1978; Martin and Benson, 1988; Suttle, 2000; Roth et al., 2008) but due to the insignificant results the importance of the study was diluted for two decades. Then, the work was mainly focused on the significance of the cyanophages in the photosynthesis of the cyanobacteria (Sullivan et al., 2006) as well as in the cycling of the nutrients in the aquatic ecosystem (Abedon et al., 2009). Their interaction with the cyanobacteria has gained tremendous importance due to cyanobacterial oxygenic photosynthesis and nitrogen fixation. This cyanobacterial-cyanophage association represents an ideal model system for studying the mechanism of oxygenic photosynthesis under viral infection and also the correlation between the viral abundance and the surrounding environment. The cyanophages infected cyanobacteria were also isolated from sea water (Proctor and Fuhrman, 1990; Suttle et al., 1990, 1992).

Apart from being the first oxygenic photosynthesizers, cyanobacteria are one of the most important and significant nitrogen fixers and primary producers. Their relationship with the cyanophages must hence be reviewed at each and every possible level so that our understanding of this cyanophage-cyanobacterial system is up to the mark. This review traverses through evolution and diversity of the cyanophages, the interaction between cyanophages and cyanobacteria, the regulation of lytic and lysogenic cycle, cyanophage development and its consequence on cyanobacterial metabolism, environmental factors affecting cyanophage development, molecular genetics of the cyanophage cyanobacterial relationship and finally, the significance of the cyanophages.

**EVOLUTION AND DIVERSITY OF CYANOPHAGES**

Till date, the actual time of the origin of the cyanophages is a matter of debate and the evolutionary studies are generally based on the cyanophageal morphology, its host specificity, genome size and its relatedness towards the highly diversified phage instead of the environment (Luftig and Haselkorn, 1967; Cowie and Prager, 1969; Chen and Lu, 2002). There have been reports of the homology among the highly conserved genes, that is, deoxyribonucleic acid (DNA) polymerases of the host and cyanophage (Braithwaite and Ito, 1993). The cyanobacteria (first oxygenic photosynthesizers in the planet) might have evolved before other bacteria and the eukaryotes that’s why cyanophages were considered as the possible original predator of cyanobacteria (Schopf and Packer, 1987; Woese, 1987; Suttle, 2000). Horizontal gene transfer especially the photosynthetic genes, was reported through infection of cyanophages on Prochlorococcus (host-strain specific) or closely related marine Synechococcus (Sullivan et al., 2006). But still the enormous diversity of the genetic material of the viruses showed a polyphyletic origin whereas the three principal families of the tailed cyanophages; Podoviruses, Myoviruses and the Siphoviruses that infect bacteria, archaea and even non filamentous and filamentous cyanobacteria are monophyletic in origin (Ackermann and DuBow, 1987b). Although the cyanophages seem to have arisen before the cyanobacteria but still the present day representatives of cyanophages; the Podoviridae, the Myoviridae and the Siphoviridae seem to be of later origin. But all these evolutionary findings are mere speculations that need to be justified by extensive morphological, biochemical and molecular characterization of the cyanophages.

From the time of discovery of the cyanophages, the nomenclature of the cyanophage was not sufficient enough to give an idea about the host, relatedness amongst the phages and the taxonomic position of the phage. For example, N-1 and AN-10 were cyanomyoviruses while N-2 and AN-9 were cyanopodoviruses (Suttle, 2000). The taxonomy of cyanophages was proposed by Safferman et al. (1983) and also was extensively reviewed by a number of workers (Desjardins and Olson, 1983; Ackermann and DuBow, 1987a). The members of tailed cyanophages having ds-DNA are classified on the basis of the contraction and size of the tail; Myoviridae, Siphoviridae and Styloviridae and are known to infect bacteria, archaea, unicellular and filamentous cyanobacteria (Ackermann and DuBow, 1987b). The cyanophages show extreme diversity in their structure, habitat and host range. They were differentiated into three major groups on the structural basis (Figure 1):

1 **Cyanomyovirus** were the first isolated, physically robust, remarkably versatile and easily adaptable viruses belonging to the Myoviridae, are abundantly found in the marine waters and less frequent in the fresh waters ((Safferman and Morris, 1963; Desjardins and Olson, 1983; Suttle and Chan, 1993; Waterbury and Valois, 1993; Wilson et al., 1993; Tetart et al., 2001, Mann et al., 2005). Some cyanomyoviruses infect fresh watered unicellular and filamentous cyanobacteria (Safferman et
Variations have also been found at the genetic and molecular level. The G+C content (mol %) of cyanomyoviruses was varied from 37 to 55% while the genome molecular weight varied from $24 \times 10^6$ to $57 \times 10^6$ Da (Adolph and Haselkorn, 1971; Safferman et al., 1972; Sherman and Connelly, 1976).

2 Cyanopodoviruses, also known as Podoviridae, have been isolated from both fresh water and marine waste stabilization ponds (Safferman et al., 1969a; Singh and Singh, 1967; Mendzhul et al., 1974; Gromov, 1983; Hu et al., 1981). LPP-1 is a cyanopodovirus that infects three different genera of non-heterocystous filamentous cyanobacteria, *Lyngbya, Phormidium, Plectonema* (Schneider et al., 1964) and shows similarities with the order Oscillatoriales on the basis of the base ratio (46% G+C) and density (1.705g/cm$^3$) (Edelman et al., 1967; Padan and Shilo, 1973). They have an isometric head (around 59 nm in diameter) with a tail (15-20 nm long) about 10 structural proteins and the genome ($27 \times 10^6$ Da) with the mol % G+C varying from the low 50s in LPP-1 (Luftig and Haselkorn, 1967) to the mid 60s in SM-1 (Mackenzie and Haselkorn, 1972a).

3 Cyanostyloviruses also known as Styloviridae, denote the *Synechococcus* infecting S-1 as the type species. It has an isometric head (about 50 nm in diameter) and a rigid tail (140 nm) which may reach up to 200 to 300 nm (Gromov, 1983; Suttle and Chan, 1993; Sode et al., 1994). There have been reports of wide variations in the number of structural proteins which may vary from 13 to 23 (Adolph and Haselkorn, 1973; Wilson et al., 1993). In comparison to the cyanomyoviruses, the mol % G+C contents reported for these was typically higher (ca. 60 to 74%) (Adolph and Haselkorn, 1973; Khudyakov, 1977; Benson and Martin, 1984).

On the basis of host range, the cyanophages have been categorized into three families (Figure 1):

A. LPP Group cyanophages which attack three different genera of non-heterocystous filamentous cyanobacteria; *Lyngbya, Phormidium, Plectonema* (Safferman and
Abedon, 2009) are the lytic cycle and the lysogenic cycle (Hanlon, 2007; Peduzzi and Luef, 2009; Hyman and Abedon, 2009). We would discuss the lytic cycle in conjunction with the lysogenic cycle in this portion of the text focusing on the control mechanism of this intricate pathway of gene regulation and expression. The cyanophages show stark resemblance to the T phages in terms of morphological attributes and also the life cycle pattern (Paracer and Ahmadjian, 2000). This resemblance in the life cycle has shaped the blueprint for much of the reports and facts that would be presented in this text.

The lytic cycle ends with the lysis and the death of the host cell. The lytic cycle depends a lot upon the activity and the effectiveness of the enzyme lysozyme that is produced by the cyanophage itself. In case of non-production of lysozyme, it has been seen that the host’s respiration and metabolism are affected and may also be ceased, but it is quite sure that the process of lyses does not occur in any case (Herskovitz and Hagen, 1980) unless and until induced. There have been a lot of studies regarding the triggering of lyses prematurely in different conditions like anaerobiosis or energy uncouplers or the protein synthesis inhibitors (Bisen et al., 1985; Wilson et al., 1993; Sode et al., 1997; Williamson et al., 2002; Boaz et al., 2006). It has been reported that UV, mitomycin C and heavy metals such as copper, cadmium can induce the release of cyanophage in marine water The five distinct stages of the lytic cycle are as follows:
Attachment

Attachment site of the phage attaches to a complementary receptor site on the cyanobacterial cell through a chemical interaction in which weak bonds are formed between the sites. Normally the presence of integrase enzymes denote the functioning of temperate life style of the cyanophages. Reports have shown that immunoglobulin-like (Ig-like) domains occur quite frequently on the surface of tailed dsDNA bacteriophages. Many of these Ig-like domains are added to cyanophage structural proteins via well coordinated ribosomal frameshifftes (Fraser et al., 2007).

Their evolutionary patterns suggest that they can be exchanged by horizontal gene transfer, independently of the protein to which they are attached in the host cyanobacteria. These Ig-like domains on the cyanophages interact with carbohydrates on the cell surface and thus facilitate phage adsorption. Also, these Ig-like domains appear to be one of a number of conserved domains displayed on phage surfaces that serve to increase infectivity by binding to or degrading polysaccharides. Without any doubt, it is a well understood fact that the tail of cyanophages is an important structure that helps in the attachment process. Studies on the bacteriophage T4 (Rossmann et al., 2004) have shown the presence of a contractile sheath that surrounds a rigid tube and then terminates in a multiprotein baseplate, to which a mesh of long and short tail fibers of the phage are attached. These fibers assume importance as they are crucial in binding to the cell receptors. The binding of these fibers to their cell receptors, leads to a conformational change in the base plate which, then initiates the contraction of the sheath and finally culminates in transfer of the phage DNA from the capsid into the host cell through the tail tube. The structure of the base plate is a dome shaped six fold symmetric structures that are stabilized by a garland of six short tail fibers, running around the periphery of the dome.

Penetration

Phage particle functions like a hypodermic syringe to inject its DNA into the bacterial cell. Although much proved reports about the cyanophages is still not present but still tailed bacteriophages are quite adroit and efficient in their ability to infect their hosts, and due to this reason they are also the most studied ones. It is basically a kind of signal transduction mechanism in which the signal is transmitted from the receptor binding site of the tail to the connector or the baseplate, causing the opening to allow the DNA ejection (Huici et al., 2004). Since it is an energy dependent process, hence it is assumed that the energy required for DNA ejection from the head is provided mainly by the DNA pressure built inside the capsid, in which it is spooled in a quasicrystalline packing (Cerritelli et al., 1997). However, this often considered a simple mechanism is far more complex, as there is not a unique way for DNA injection and it has been found to differ amongst the different hosts and the phages also. While the above process is considered to be active and energy dependent in case of Bacillus subtilis phage φ 29, it has also been proposed that the host metabolic machinery does not have any significant role or infact plays no role in the process of injection of the phage DNA into the host cell in case of E. coli (Maltouf and Labedan, 1985). Experiments conducted by workers throughout the world have indicated that even the use of metabolic inhibitors and uncouplers have not been able to disrupt or delay the first step transfer of the DNA segment. Hence it has also been proposed that the transfer of the DNA across the cytoplasmic membrane or the surrounding membranes of the host cell happens by the process of diffusion across the protein channels of the host cell (Maltouf and Labedan, 1983). Dusenbery and Uretz (1972) reported about the inhibition of bacteriophage DNA injection by dyes bound to the DNA, like proflavine, acridine orange, or ethidium, but not polyamines. It seems that the presence of the dye is required inside the permeability barrier of the phage at the time of infection. The effect of proflavine is reduced in the presence of polyamines, suggesting that the active site is certainly on DNA. With the different modes of penetration, the rate of injection of this process also varies a lot. One of the fastest studied modes of injection has been observed in the phage T4 in which 169 Kb genome is transferred in about 30 s through the help of the protonmotive force (Boulanger and Letellier, 1988; Letellier and Boulanger, 1989; Huici et al., 2004) across the ion channels in the cytoplasmic membrane.

Biosynthesis

Initially the phage uses the host cell’s nucleotides and several of its enzymes to synthesize many copies of phage DNA. Later, the biosynthesis of phage proteins begins. Any RNA transcribed in the cell is mRNA transcribed from phage DNA for the biosynthesis of phage enzymes and DNA for the biosynthesis of phage enzymes and capsid proteins. The host cell’s ribosomes, enzymes and amino acids are used for translation. For several minutes after infection, complete phages cannot be found in the host cell. Only separate components-DNA and protein-can be detected. The period during phage multiplication when complete and infective phages are not yet present is called the Eclipse Period (Amla, 1981).

Maturation

Assembly of the different components happens in the
maturation phase. All the important proteins that are needed for the phage progenies to survive outside the host cell are manufactured and assembled into their respective sites inside the phage genome (Moisa et al., 1981b). On a whole, the late messenger ribonucleic acid (mRNA) synthesized in the biosynthesis stage directs the synthesis of three kinds of proteins: phage structural proteins, proteins that help with phage assembly without becoming part of the virion structure, and proteins involved in cell lysis and phage release (Barrell and Air, 1976; Pollock et al., 1978; Atkins et al., 1979; Beremand and Blumenthal, 1979; Bernhardt et al., 2002). At the time of packaging and assembly of the phage components, the DNA is translocated into already preformed protein shells. During the process of packaging, irrespective of the type of cyanophage or bacteriophage, there is a change in the conformation and the properties of DNA (Rao and Feiss, 2008). As a result of massive compaction and compression, the highly negatively charged DNA is brought at the same density as that of crystalline DNA (Earnshaw and Casjens, 1980; Rao and Feiss, 2008). This process of packaging is not a spontaneous process, but is powered by adenosine triphosphate (ATP) hydrolysis accomplished by the help of a translocating motor. This translocating motor is actually responsible for the compaction of the DNA molecule into a compact shell. This preformed shell is usually an icosahedron that is formed by many copies of the major capsid protein. The process of translocation is achieved by the enzyme terminase (Szpirer and Brachet, 1970; Rao and Feiss, 2008). It is basically an amalgamation of hetero oligomers of a small protein involved in DNA recognition and the larger protein involved in translocation ATPase, an endonuclease enzyme and a motif for docking at the portal vertex (Black, 1989; Rao and Feiss, 2008). The endonucleases are used in the cutting of the large concatemers of DNA into the smaller lengths. The ATPase associated with the terminase assists in the powering of the translocation machine. The smaller subunit associated with the terminase becomes very useful during the DNA recognition and the differentiation of the phage DNA from the host DNA (Rao and Feiss, 2008).

Release

The mature phage particles are released after the lyses of the cyanobacterial cell. The process of release is important as the cyanophages need to escape from the host cyanobacteria and let their new progenies be dispersed so that more of the host can be parasitized (Knobler and Gelbart, 2009). This process though sounds simple, is associated with a lot of complexities for the new virions as the cell wall of the bacteria is nearly impregnable for the phage particles (Tzlil et al., 2003). The primary obstacle encountered is the highly sophisticated and compact continuous meshwork of the peptidoglycan layer which nearly renders the outer surroundings out of bounds for the newly formed phage particles. Even the huge amount of freshly formed phages is unable to build up sufficient pressure for even the osmotic break up of the peptidoglycan layer (Young et al., 2000). Hence, in order to escape out of the cell wall the phage needs specialized mechanisms for lysing the cyanobacterial cell wall. Amongst the strategies known and observed till now, two are particularly important (Young, 1992; Young et al., 2000). The phage may proceed with release process either through the endolysin mediated mechanism or through the holing mediated lysis (Wang et al., 2000; Mann, 2003). The use of the above two mechanisms has been observed in most of the phage particles with the use varying to different extents (Mann, 2003). The need for the holin mediated pathway is due to the fact that endolysins lack a secretory signal sequence. Hence the endolysins need the assistance of holins to gain access to the murein of the cyanobacterial cell wall (Mann, 2003). The holins have tremendous capacity to somehow pemeabilize the membrane and thus assist the endolysin in the phage release process (Young et al., 2000). Hence they are important in the regulation of the timing of the process of lysis and release. The endolysin accumulates in the cytosol but even this is not sufficient enough to attack the murein layer (Young et al., 2000). The holin, at a genetically programmed moment, forms a lethal membrane lesion that terminates respiration and thus allows the endolysin to get access to the murein layer. The term “lesion”, here denotes simply a kind of hole that allows the endolysin to cross the cytoplasmic membrane (Young et al., 2000).

THE CHOICE FOR LYTI C CYCLE OR LYSOGENIC CYCLE

The lytic lysogeny pronouncement has long been regarded as a standard for the works in developmental genetic networks. This intricate understanding is exemplified by three key features and their operation in different conditions. Firstly, it depends on the environment signals and the extent of phage infection per cell which cycle has to be operated. Secondly, the stability of the lysogenic prophage is without any doubt, very high. Thirdly, the highly stable lysogenic prophage enters the lytic cycle swiftly when it encounters conditions that are detrimental to them or to their genetic material. This means that the decision that whether lytic cycle or lysogenic cycle has to be operated depends upon the nutritional status or the presence of any pathogenic condition to which the bacterium is susceptible. The CI and the Cro proteins embody the bistable genetic switched regulators that are responsible to a large extent for the functioning of any of the above cycle. While the CI protein is associated with
the maintenance of the stable lysogenic phase, the Cro protein sets the lytic cycle into action by lowering the level of clear II (CII) protein that in turn fails to activate the expression of the CI protein. This regulation is governed by the combined and synchronized action of a large number of proteins and promoters that are responsible for the lytic or lysogenic decision. The decision is hence not dependent upon a single individual factor or protein but instead relies on a large number of factors and their coordination with each other. The various proteins and the promoter’s along with their structural and functional aspects were discussed in the coming sections in depth and detail. The CI Protein encodes the Lambda repressor (Figure 2), a protein of two domains joined by a flexible linker region of about 40 residues (Pabo et al., 1979). Here the N-terminal domain, residues 1-92 are responsible for DNA binding whereas the C-terminal domain, residues 132-236 participate in dimerization (Bell et al., 2000; Friedman and Court, 2001). The process of dimerization is important as the binding to the operator needs the dimeric form so that the two DNA binding domains can contact the operator simultaneously (Bell et al., 2000). This is important as although the information for DNA binding lies only in the N-terminal domain but the efficiency of the process is greatly enhanced by the attachment and dimerization of the C-terminal domain. The dimeric structure of the repressor is very crucial for maintenance of lysogeny as it is the crucial connector between the two monomers whose lysis is responsible for the induction of the lytic cycle (Carlson and Koudelka, 1994). The connection between the residues 111 and 113 is broken down and this is the phase when the lytic cycle is induced. The factors responsible for induction may be a variety of adverse conditions like UV irradiation or any pathogenic condition which ultimately leads to the proteolytic inactivation of the repressor. On induction of the lytic cycle, the connection between the dimers is cleaved and only the N-terminal domain remains in the monomeric form. This type of formation upset the equilibrium obtained in the dimeric state. Due to this, the repressor dissociates from DNA, thus starting the lytic cycle. Also, the disturbed dimeric state upsets the cooperativity between the adjacent dimmers and thus this also contributes to the induction of the lytic cycle. The binding to the DNA done by the repressor dimer through the central base pair is recognized. Half sites are also present on each side of the central base pair and thus each N-terminal region contacts a half site. The N-terminal domain of the lambda repressor consists of several stretches of α-helix. Out of the total five stretches present, two of them are responsible for binding DNA. The type of structural arrangement is called a helix-turn-helix model. In each monomeric form, the α-helix-3 consists of nine amino acids which lie at fixed angle to seven amino acids of the α-helix-2 (Branden and Tooze,
This strategic placing of the α-helices places the two helix-3 regions at a distance of 34 Å and thus makes them suitable to fit into the major grooves of DNA. In the dimeric form, the helix-2 regions lie at a point that places them across the grooves (Branden and Tooze, 1999; Kenny et al., 2006). This symmetrical positioning of the dimer to the sites ultimately causes the binding of each N-terminal domain of the dimer to a similar set of bases in its half site. Now the question that may come to the mind of most of the readers would be to define the role of both the helices and their importance in this context. The helix-3 is responsible for making contact between the exposed hydrogen bonds of the amino acid side chains and the exposed part of the base pairs. This contact makes the recognition of the appropriate part of the DNA double helix possible (Branden and Tooze, 1999; Kenny et al., 2006). Hence this helix is also known as the recognition Helix. The helix-2 contacts the phosphate backbone through the hydrogen bonds. This serves the purpose of binding although it seems to have no role in specifying the recognition of the target sequences (Branden and Tooze, 1999; Kenny et al., 2006). It represses $P_R$ and $P_L$ which are termed as the major rightward promoter and major leftward promoter. It also interacts with $P_{RM}$ and this interaction depends on the concentration of the CI protein itself (Dodd et al., 2005; Kenny et al., 2006). If the concentration of the CI protein is high then it represses $P_{RM}$. At low concentrations, $P_{RM}$ is activated by CI protein (Dodd et al., 2005; Kenny et al., 2006). The Cro Protein is an imperative protein which is known to prevent the synthesis of the repressor and secondly it also turns off the expression of the immediate early genes. Thus, through its dual functions, it finally leads to the repression of lysogeny and establishment of lytic cycle. The CII protein is the activator of $P_{RE}$ and $P_{int}$. The N protein functions as an antiterminator factor. The N protein, just like Cro, is a decisive lytic regulator but acts by a completely different mechanism. It antiterminates transcription at termination signals, allowing expression of distal genes in the $P_L$ and $P_R$ operons (Oppenheim et al., 2005). Its action at the nut sites allows transcription to proceed into the delayed early genes (Court et al., 2007). The Q protein also functions as an antiterminator factor that is responsible for mediating the transcription of late genes through the host RNA polymerase (Court et al., 2007). The CIII protein stabilizes/protects the CI protein. The above proteins help in deciding the fate of the cell that whether it would go for lytic cycle or lysogenic cycle. Alongwith these proteins, a number of promoters are also needed to aid and assist the proteins in the decision making process viz., $P_L$: major leftward promoter; $P_R$: major rightward promoter; $P_{RE}$: promoter for repressor establishment; $P_{RM}$: promoter for repressor maintenance; $P_{int}$: promoter for integration; PR: secondary rightward promoter.

After this detailed discussion about the proteins and the promoters, one fact that becomes highly significant and imperative is that the decision for lytic cycle or lysogeny depends on the relative concentration of either the Cro protein or the CI protein (Dodd et al., 2005; Kenny et al., 2006). Hence the ratio of the above two proteins seems to play one of the most important roles in the regulation of lytic and the lysogenic cycle. Alongwith the Cro protein and the CI protein, their affinities with the $O_R$ protein is important. The $O_R$ has three sites viz., $O_{R1}$, $O_{R2}$, and $O_{R3}$ (Court et al., 2007). The CI proteins order of affinity towards the $O_R$ sites is $O_{R1}$ > $O_{R2}$ > $O_{R3}$. Similarly, the Cro proteins order of affinity towards the $O_R$ sites is $O_{R3}$ > $O_{R2}$ > $O_{R1}$. If CI binds to $O_{R1}$ then $O_{R2}$ and then RNA polymerase cannot bind to $P_R$ and genes on $P_R$ cannot be synthesized. Hence lytic cycle does not happen (Figure 3).

**THE CONTROL OF LYTIC AND THE LYSOGENIC CYCLE**

The basic strategy of the phage is always to incorporate the least number of genes and proteins that are needed inside the cyanobacteria and thereafter to insert these at strategic places of need. The phage genome is itself of a very small size and hence proper selection of the genes and proteins is very much necessary. As a means of survival, the phage does need three imperative phases: replication, transcription and translation. As far as the replication of the DNA is concerned, the phage relies on its own genes that are needed for replication inside the host. These genes are accountable for the initiation of replication and may even include a new DNA polymerase. Coming to the transcriptional level, the preferential transcription of the phage mRNAs is the basic driving force and it is accomplished by either the changes at the host cell or substitution of the RNA polymerase or even modification at the initiation or the termination level. During the process of protein synthesis, the phage relies a lot on the host protein synthesis apparatus except that the cyanobacterial mRNA is replaced by the phage mRNA. The development and the expression of the phage genes always happen in a sequential manner in which there are basically two phases of expression of the genes: the early phase and the late phase. The genes of the early phase are concerned with the production of enzymes involved in the reproduction of DNA (Eisen et al., 1968; Young 1992). These are needed for DNA synthesis, recombination and sometimes even modification. All these fast paced activities of these genes lead to an assembly and accretion of genes and gene products. Now during the late phase, the assemblage of the protein components of the phage starts. In this phase, both the structural and the assembly proteins are needed by the phage particle. The assembly proteins are needed for the construction of the phage particles although they themselves are not
incorporated in the genome of the phage. Now it must be kept in mind that the starting point for both lytic or the lysogenic cycle is the same. The pathway for both the cycles is sort of interlocked with each other. The lysis-lysogeny pronouncement is subjected by the number of phage particles infecting the cell as well as by the cell physiology (Kourilsky, 1973; Kourilsky and Knapp, 1974; Oppenheim et al., 2005). High multiplicity of infection is known to favor lysogeny. Also the nutritional status of the cells that has been discussed earlier is important. In nutrient rich media, the lytic course predominates, whereas growing cells in poor media increases the chances of the lysogenic pathway after single infection (Kourilsky and Knapp, 1974). Cells raised in nutrient rich media have higher concentration of the host global regulator RNase III, which leads to elevated rates of expression of the protein N favoring lytic growth (Wilson et al., 2002). In carbon-starved cells, on the other hand, RNase III and consequently N concentrations are low. Under these conditions N translation is repressed. This reduction of N concentration would reduce Q expression to a level that provides more opportunity for lysogenic response. When the phage DNA enters the host cell, then there are options for the operation of both lytic and
the lysogenic cycle. Both the processes require the synthesis and also the expression of the immediate early and the delayed early genes. But after this point, there occurs the point of deviation of both the processes: lytic process occurs if the late genes are expressed and lysogeny happens if the repressor gets expressed.

**ENVIRONMENTAL FACTORS AFFECTING CYANOPHAGE-CYANOBACTERIAL INTERACTION**

Being a component of the ecosystem, it is natural that cyanophages do get affected immensely by the changes in environmental conditions (Shilo, 1972; Jessup and Forde, 2008). This section would deal one by one with the possible factors that may affect the sustenance of the cyanophages (Figure 4).

**Temperature**

It is one of the most important factors that affect the stability of phages. Most of the reports regarding the effect of temperature on the cyanophages have been from isolates and only few from naturally occurring cyanophage communities. Temperature is an imperative inducer for AS-1 cyanophage conversion from lysogenic to lytic cycle and could have applied significance in the modulation of cyanobacterial populations in freshwater aquatic environments (Chu et al., 2010). Although freezing affects the cyanophages yet in genera, cyanophages can be stored for a month or even more than 4°C (Desjardins and Olson, 1983; Safferman and Morris, 1964b; Safferman et al., 1969b). It was shown that at higher temperatures it depended upon the thermo-tolerance capacity of the cyanophages; whether they
could survive or not. The thermally tolerant strains survived at greater than 40°C (Safferman and Morris, 1964b; Safferman et al., 1969b) while the sensitive ones were unable to survive and no plaque formation was seen even at 35°C (Padan et al., 1971). Above 50°C, there was a loss of infectivity at the temperatures (Safferman and Morris, 1964b; Padan et al., 1971; Sarma and Singh, 1995).

Solar radiation

The intense solar radiation on the infectivity of the cyanophages has been studied and is one of the major causes that accounts for the loss of the phages in the natural environment (Suttle and Chen, 1992; Wommack et al., 1996; Nobel and Fuhrman, 1997; Wilhelm et al., 1998). Solar powered circulation (SPC) has been recently reported to be a cure for suppression of freshwater harmful algal blooms (Hudnell et al., 2010). The rate of removal of the natural phage communities were measured to be 0.53 and 0.75 day⁻¹ when they were integrated over the surface mixed layer and subsequently averaged over 24 h. It is well known that the rate of decay varied inversely with the depth. It seems that the decay rates also depend on the season.

According to a general consensus, phage communities are more resistant to damaging effects of sunlight in the summers as compared to the springs and the winters. When the natural phage communities and isolates were compared, decay rates of the isolates were about 2-fold higher than the rate of natural phage communities in summers. The loss of the infectivity of the phages in sunlight is the result of the pyrimidine dimer formation. It is well known that cyanobacteria have a very efficient DNA repair mechanism and the same is also helpful repairing the damaged phage DNA (Wu et al., 1967; Singh, 1975; Asato, 1976; Amla, 1979; Hwang-Lee et al., 1985; Levine and Thiel, 1987). It is very clear that the damage due to the infectivity is basically based on the DNA damage and not in the adsorption or injection of the phage DNA into the host. The environmental factors that can influence the phage infectivity and the survival in sunlight are solar angle and insolation, water transparency and depth of the mixed layer (Suttle, 2000).

Cation concentrations

The concentration of cations seems to influence the cyanophage infectivity a lot. A specific or universal response is not associated towards the effect of cations on the cyanophages. There are many cases where there is no effect of the cations while there may be cases where the cation concentrations may play an important role. Presence of Mg²⁺ is reported to play an important role in maintaining the integrity of the phage capsid of lipid phosphate phosphatase-1 (LPP-1) and in their stability in comparison to normal deionized water (Safferman and Morris, 1964b; Schneider et al., 1964) and SM-1 (Martin and Benson, 1988). At or above 1mM Mg²⁺, the phage remained infective while at 0.1mM concentrations, the phage lost its infectivity (Goldstein et al., 1967).

Also AS-1 was reported to be more stable in the presence of cations (Amla, 1981; Desjardins and Olson, 1983). An important conclusion that could be drawn from the above three reports is that even though the three phages belong to three different families all of them need cations for sustenance. Hence a generalized concept about the need of a particular cation for a particular taxonomic group cannot be made. In addition to these reports about the need of cations, there are reports about the phage SM-1 that has no cation requirements and they remain infectious when resuspended in distilled water (Safferman et al., 1969b). Other cations that are reported to play important roles for phage infectivity are Mn²⁺ and Na⁺ (Amla, 1981). An interesting observation made by Bancroft and Smith (1992) was that the cyanophage AS-1 could not tolerate NaCl concentrations above 0.05M.

pH

There seem to be no clear cut and conclusive reports about the effect of pH on the sustenance of infectivity of cyanophages. It is well known that cyanophages can tolerate wide variations in the pH and hence this may not be a very important factor in the distribution of phages. Freshwater cyanophages are known to have greater pH tolerance than other bacteriophages and the later lose their infectivity at pH greater than 8 or 9 (Adams, 1959; Ackerman and DuBow, 1987a). LPP-1, AS-1 and SM-1 showed pH range from pH 5 to 11 (Safferman and Morris, 1964b; Safferman et al., 1969b, 1972). Hence in terms of pH tolerance, cyanophages show better adaptivity than the bacteriophages and its broad pH tolerance also reflects the pH of their hosts, that is, the cyanobacteria can tolerate.

Salinity

Little information is gained regarding the effects of salinity on cyanophages distribution. Most of the studies only are focused only on marine ecosystems rather than fresh water systems. Lu et al. (2001) studied the effects of salinity on the distribution, isolation, host specificity and diversity of cyanophages and reported that salinity play a significant impact on both the cyanophage or host population structure. The growth of different strains of Synechococcus was studied at different salinities. It was observed that PE strains did not grow well when the salinity of the medium was less than 28% while the PC
strains grew well at salinities ranging from 18 to 30%. Thus, on the basis of the above results it could be concluded that the PC strains had much better capacity to adapt at high salinity than the PE strains. Another interesting inference that could be drawn from the above work was that the cyanophages that resided at high salinity water (coastal water) were had better and much more diverse morphotypes than those which inhabited low salinity water (upstream water). At the end, Lu et al. (2001) concluded that the abundance of cyanophages on *Synechococcus* spp. was directly proportional to the salinity in three Georgia coastal rivers.

**Depth**

The depth of the water column has significant impact on the planktonic life especially that of the cyanophages. Various cyanophages having different hosts show different preferences for water depth. It is obvious that water temperature and salinity gradients vary gradually with the depth of the water column. This variation would certainly lead to a kind of stratification, ultimately leading to different kinds of microenvironments within the same water body. Studies conducted at the Chesapeake Bay showed that during the calm summer weather, the bottom water body which was more saline, becomes anoxic and thus affected the various life forms present there (Officer et al., 1984; Tuttle et al., 1987). Work done at different sites proved that the abundance of the virioplankton significantly decreases below the euphotic zone in the open ocean water (200 m). According to estimates, the abundances can be as low as around \(10^5\) viruses per ml (Paul et al., 1991; Bird and Maranger, 1993; Boehme et al., 1993; Hara et al., 1996; Steward et al., 1996). Frederickson et al. (2003) studied the effects of the physical environment on cyanophage communities in British Columbia inlets. Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified g20 gene fragments were analyzed to examine the variations that were encountered in three inlets. According to their observations, some of the cyanophages were very abundant and were found in the entire sample while some were found only at specific depths. Above variation certainly reflected that some phages show preferences for certain depths in a water column. This may be due to the salinity or the temperature gradients at the depths. At greater depths, the phages show a decline in their abundance and a sort of preferential occurrence.

**Seasonal variations**

Seasonal variations influence the abundance of the cyanophage and the existence of viruses in seawater. It certainly affects the distribution of the phages. The concentration of viruses was reported to fall from about \(5 \times 10^6\) per ml to \(10^4\) per ml during winter seasons in the Norwegian coastal waters. Viral abundances shifted significantly during the winter and the summer seasons in the Tampa Bay and the Northern Adriatic Sea. From the low levels in the winters (\(10^6\) per ml) to the summer highs of around \(10^7\) per ml or more, the shifts were certainly significant (Jiang and Paul, 1994; Weinbauer et al., 1995; Cochran and Paul, 1998). Around \(12 \times 10^6\) to \(61 \times 10^6\) per ml of viral particles are reported from the freshwater ecosystems in the Danube River (Mathias et al., 1995). Normally, the viral abundance is at its peak in the autumn and the spring season but the peaks in the autumn season show greater viral abundances than the spring season. In the coastal waters of the Rhode Island, temporal variations of cyanophages were reported by Marston and Sallee (2003). Extinction dilution enrichment and restriction fragment length polymorphism (RFLP) were used to detect the relative abundance of cyanophages in June, July and August, 2002 and observed result showed that the abundance of cyanophages varied according to season and specific cyanophages were dominant in different seasons. Apart from the above listed factors there are more factors which may certainly affect the cyanophages abundance at different locations and at different times. But, the above were some of the most important factors that cause changes in the abundance, distribution and diversity of cyanophages.

**MOLECULAR GENETICS OF THE CYANOPHAGE CYANOBACTERIAL INTERACTION**

Recent works and observations suggested that cyanophages directly control the photosynthetic behaviour of the cyanobacteria. *Prochlorococcus* and *Synechococcus* are important in light of their share in the process of global carbon fixation. It is estimated that they account for nearly 30% of the fixed carbon around the planet. Like the other photosynthesizers they also use the photosystems I and II for carrying out their photosynthesis. It has been reported that cyanophages not only possess genes for photosynthesis but are also involved in the exchange of genetic material with the cyanobacteria (Sullivan et al., 2006). The two core photosystem II reaction centre proteins D1 and D2 are encoded by the *psbA* and *psbD* genes. The *psbA* is one of the most rapidly turned over core protein in all oxygenic evolving photosynthesizers. The *psbD* is involved in a kind of protein complexation with *psbA*. During stress, D1 is rapidly turned over. This also happens during phage infection (Falkowski and Raven, 1997). The phages switch on its lytic cycle in the presence of any kind of stress, that is, it needs to do phage gene transcription rather than the supporting host gene transcription. The *D1* genes continue to produce the protein which helps in
maintaining the process and level of photosynthesis (Mann et al., 2003; Lindell et al., 2004, Millard et al., 2004). Since D1 is encoded by psbA, hence the essential role of psbA during infection is beyond any doubt and cannot be questioned (Lindell et al., 2004).

Moreover, evidence has emerged from the ubiquity of psbA amongst the cyanophages, their similarity to cyanobacterial hosts and at last the conserved amino acid sequences. It has been reported that although the psbA and psbD genes are wide apart on the cyanobacterial genome they are very close to each other on the cyanophage genome. In context to the algal-cyanophage photosynthetic machinery, it has been reported that 88% of the cyanophages of Sargasso Sea and the Red Sea possess psbA while 50% had psbD (Sullivan et al., 2006). psbA was also found in all myoviruses and Prochlorococcus podoviruses. Although psbD was not present in all and it was less prolific but still it was present in some cases; 20 Prochlorococcus myoviruses and 17 Synechococcus myoviruses. Another interpretation of the findings is that in cases where psbA was present, psbD may or may not be present but in cases where psbD was present psbA was undoubtedly present. The myoviruses cyanophages have the maximum abundance and the broadest host range. Later on, a heterodimer protein complex was formed by psbA-psbD which had the broadest host range. Thus it seems that this complex formation is involved in charge separation which tends to help the cyanophages for better infectivity by diversifying their host range. There is the existence of a lot of conserved sequences in the cyanophages and these sequences help the phages to acclimatize and infect in a much better way. The source and the time of transfer of the PSII genes into the cyanophages was also investigated (Sullivan et al., 2006). Nucleotide sequences of psbA and psbD from Prochlorococcus and Synechococcus were analyzed by sequencing and the use of statistics which clearly indicated horizontal gene transfer where psbA was transferred four times from the cyanobacteria genome to the cyanophage genome. The transfer was two times for psbD from the cyanobacteria to the cyanophage. The exchange was generally host specific but cases where these generalizations were not followed were also seen. These exceptions indicate towards a type of cross-exchange between two different phages during co-infection of the same host. This intragenic recombination seems to favour the cyanophages in their efficiency of cross-infectivity. Also, this gene-shuffling helps the phage to efficiently co-ordinate with the photosynthetic machinery of the host cyanobacteria especially during the stress conditions when the lytic cycle needs to be operated. The genes responsible for photosynthetic electron transport thylakoid associated genes were also transferred from the cyanobacteria to the cyanophage. The PET proteins petE and petF were found in Prochlorococcus phages (Lindell et al., 2004) and high light inducible proteins hliP were found in one case in Prochlorococcus and in one case in Synechococcus.

Astonishing were the findings that the sequences in the Prochlorococcus phages matched with the homologues found in the hosts (Lindell et al., 2004). This indicates directly that the genes are functional. Now if it is assumed that the genes are functional then they can maintain the activity of the PSII reaction centre in two ways. In case of electron deficit they can provide the back up electrons away from PSII (pet genes). They can also support the photosynthesis in plants by stabilizing the PSII reaction centre (PsBD and HliP (Lindell et al., 2004).

Thus the genetics of the cyanophages has mostly been studied by taking the photosynthetic genes as the models which revealed a lot about the evolution of the cyanophages. Recent reports have indicated the role of a certain antisense RNA (asRNA) that is co-regulated with psbA. Analysis of scaffolds from global ocean survey datasets confirms that this asRNA seems to be commonly associated with the 3′ end of cyanophage psbA genes, indicating that this proposed mechanism of regulation of marine ‘viral’ photosynthesis is evolutionarily conserved and thus gains phylogenetic eminence (Millard et al., 2010).

This process of microbial evolution has occurred through ages and certainly represents one of the best examples of horizontal gene transfer. Thus, the whole work reveals a lot about the diversity of cyanophages and their potential for evolution but still better results are needed for proper studying of the phage diversity. There is a need for better approach using proper molecular biology tools and bioinformatics. The metagenomic approach can be an ideal way for proper and complete understanding of the enormous, uncharacterized diversity of the phages. This can be done by the sequence analysis of the shotgun libraries of the total viral DNA. Chen and Lu (2002) sequenced the genome of cyanophage P60 infecting marine Synechococcus WH7803 which has 47872 bp’s with around 80 ORF’s. The podoviruses have a more conserved DNA replication system than the siphoviruses and the myoviruses. In phylogenetic analyses, the coding regions for nucleotide metabolism were very helpful having a lot of similarities with the cyanophage P60 and marine unicellular cyanobacteria. The DNA structure of LPP-3 was peculiar in having protruding 3′-ends with the ends showing no complementarity (Syrchin and Mendzhul, 2002). Restriction analysis of separate fragments has indicated the possible use of genome of LPP-3 as a new cloning vector in cyanobacterium. Reports of the similarities in sequences from g18 to g23 between the marine cyanomyovirus S-PM2 and coliphages T4 was given by Hambly et al. (2001). The order of the genes has been reported to be similar except for the presence of some insertions and deletions of small ORF’s of unknown function. The studies reported that just like the T4 phage where g18 codes for the tail sheath, g19 for the tail tube,
g20 for the head portal protein, g21 for the prohead core protein, g22 for a scaffolding protein, and g23 for the major capsid protein, the cyanophages also do not deviate on a very big scale. Thus, the differences and the similarities indicate a kind of genetic shuffling that may have occurred or is still occurring progressively by the sharing of these types of conserved modules.

SIGNIFICANCE OF THE CYANOPHAGE-CYANOBACTERIAL INTERACTION

The significance of the cyanophages was initially related to the prospects of their use in the control of algal blooms but later on it was reported that the cyanophages exhibited specificities for the cyanobacteria they predated on and it became apparent that every cyanophage cannot infect and control every cyanobacteria. Moreover some recent findings indicated the enhanced dispersal of toxic filamentous cyanobacteria on using lake side phages which further advocates towards the inefficiency of cyanophages in the control of algal blooms (Pollard and Young, 2010).

Also some fungal strains have been reported to be very competent in the degradation of phytoplankton species and thus have opened new avenues for the biological control of algal blooms (Jia et al., 2010 a,b). That is why there was a loss of interest in the studies related to cyanophages. After a long gap, they again became a hot topic of research mainly due to their enormous abundance of Synechococcus and Prochlorococcus in the marine ecosystem. Due to most significant carbon and nitrogen fixers, Synechococcus and Prochlorococcus is an important matter of research because the cyanophages are associated with them and it is speculated that there must be a kind of inter-relationship with their hosts. Research related to this association later on has led to some very appealing facts that gain prominence in view of the role of the cyanophages in the process of genetic exchange and a kind of gene shuffling.

Reports about the homology of the photosynthetic genes and the assembly genes amid cyanophages and their hosts exemplify the phylogenetic importance of the cyanophages. The enormous diversity of the cyanophages certainly indicates the exchange process that might occur with their hosts and this is a topic that needs a sound application of molecular biology and techniques along with a sound application of bioinformatics tools.

The study of photosynthesis under viral infection has been understood significantly by experiments on the cyanophage cyanobacterial system. The seasonal abundance of the cyanophages indicates their possible role in maintaining the ecological poise of the ecosystem and in the maintenance of the biogeochemical cycling and nutritional status of the surroundings. Hence grazing of the cyanobacteria by the cyanophages does help to maintain the population structure of the ecosystem keeping the nutrient cycling and microbial diversity at an appropriate level. The lytic cycle performed by different phages at different stages of their life cycle is also an important mechanism through which the balance in the population structure is maintained. Dammeyer et al. (2008) in their findings state that the cyanophages mediate the biosynthesis of pigments in the oceanic cyanobacteria. Such phage mediated biosynthesis of pigments certainly needs to be tested further so that positive results may have some biotechnological applications. This would also enhance the growing belief about the cyanophages a Living laboratory of continuously increasing discoveries. Bailey et al. (2004) in their report state that the cyanophages may help the cyanobacteria against photo-inhibition in high sunlight thereby protecting the host against the harmful aftermath of intense light intensity. This would in turn assure the cyanobacteria of a proper energy supply for processes such as replication and thus help the cell maintain its integrity. Thus it is apparent that at the present time the field of phage biology is going through a period of renaissance in which new and exciting innovative ideas are coming from all parts of the globe. There is a need for a proper understanding of these reports so that they can be implemented for the welfare of all the life forms on the planet (Figure 5).

FUTURE PROSPECTS

Cyanophages represent one of the most abundant and also the most diverse life-forms whose diversity is still untapped and uncharacterized. The genetic diversity they exhibit is simply awesome and it certainly throws a challenge to the microbiologists so that they can tap its true potential. The significance of the cyanophages gains importance in view of the horizontal gene transfer that has been reported in the case of the photosynthetic genes of cyanophages. Apart from this, reports are coming on a regular basis about the various systems where there has been a recombination or an exchange of genes between the phages and the cyanobacteria. Thus the importance of the cyanophages cannot be questioned in the field of phylogenetic and evolutionary science. Apart from this, the various roles played by the phages in bloom control, pigment biosynthesis, photo-inhibition protection and maintenance of the community structure needs to be further assessed with enhanced focus on the use of molecular biology. More and more use of specific primers along with suitable techniques like RFLP, DGGE, fingerprinting etc. are the need of the hour so that a better understanding at the physiological, biochemical, genetic and molecular level is reached. An important role can be played by the field of the new and emerging stream of molecular evolution. Better tools of bioinformatics and computational biology are needed for a sound interpretation of the field data.
Hence as a concluding note, people from physiology, genetics, microbiology, evolution, molecular biology, biochemistry and bioinformatics need to work together to explore and understand the various unknown facets of these small but immensely important group of organisms. In short, the whole of science needs to go hand in hand to understand this living laboratory of innovation.

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ABBREVIATIONS

ds-DNA, Double-stranded deoxyribonucleic acid; Cro/CI, control of repressor’s operator/clear 1; t-RNA, transfer ribonucleic acid; Ig-like, Immunoglobulin-like; mRNA, messenger ribonucleic acid; ATP, adenosine triphosphate; SPC, solar powered circulation; LPP-1, lipid phosphate phosphatase-1; DGGE, denaturing gradient gel electrophoresis; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; asRNA, antisense ribonucleic acid.

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