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## The genome of the brown alga *Ectocarpus siliculosus* contains a series of viral DNA pieces, suggesting an ancient association with large dsDNA viruses

Nicolas Delaroque\* and Wilhelm Boland

Address: Max-Planck-Institut für Chemische Ökologie, Beutenberg Campus, Hans Knöll Str. 8, D - 07745 Jena, Germany

Email: Nicolas Delaroque\* - [ndelaroque@ice.mpg.de](mailto:ndelaroque@ice.mpg.de); Wilhelm Boland - [boland@ice.mpg.de](mailto:boland@ice.mpg.de)

\* Corresponding author

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### Abstract

**Background:** *Ectocarpus siliculosus* virus-1 (EsV-1) is a lysogenic dsDNA virus belonging to the super family of nucleocytoplasmic large DNA viruses (NCLDV) that infect *Ectocarpus siliculosus*, a marine filamentous brown alga. Previous studies indicated that the viral genome is integrated into the host DNA. In order to find the integration sites of the viral genome, a genomic library from EsV-1-infected algae was screened using labelled EsV-1 DNA. Several fragments were isolated and some of them were sequenced and analyzed in detail.

**Results:** Analysis revealed that the algal genome is split by a copy of viral sequences that have a high identity to EsV-1 DNA sequences. These fragments are interspersed with DNA repeats, pseudogenes and genes coding for products involved in DNA replication, integration and transposition. Some of these gene products are not encoded by EsV-1 but are present in the genome of other members of the NCLDV family. Further analysis suggests that the *Ectocarpus* algal genome contains traces of the integration of a large dsDNA viral genome; this genome could be the ancestor of the extant NCLDV genomes. Furthermore, several lines of evidence indicate that the EsV-1 genome might have originated in these viral DNA pieces, implying the existence of a complex integration and recombination system. A protein similar to a new class of tyrosine recombinases might be a key enzyme of this system.

**Conclusion:** Our results support the hypothesis that some dsDNA viruses are monophyletic and evolved principally through genome reduction. Moreover, we hypothesize that phaeoviruses have probably developed an original replication system.

### Background

For several years, the investigation of novel dsDNA viruses has excited the imagination of virologists and evolutionists, prompting the development of several original theories about the evolution of certain dsDNA viruses and their role in shaping the genome of the organisms of the tree of life. It has been proposed that dsDNA viruses were

the evolutionary origin of the eukaryotic nucleus or at least that they have contributed to its creation by transferring genetic information to the nucleus [1-7].

The recent discovery and characterization of several large dsDNA viruses from aquatic environments belonging to the phycodnavirus and mimivirus families led to the

development of new hypotheses [8-10]. A comparative analysis of the gene content of these viruses with poxviruses, iridoviruses and asfarviruses indicated that they have nine gene products in common, and 33 more gene products are present in at least two of these families. It follows that they might have a common evolutionary ancestor, a nucleocytoplasmic large dsDNA virus (NCLDV) [11,12]. Although most evolutionists seem to accept the hypothesis of a common ancestor, there is no consensus concerning the general morphology of the ancestral NCLDV and how it evolved to give rise to the different classes of viruses. On the one hand, the ancestral NCLDV is believed to have been a dsDNA virus which has evolved by acquiring genes from the host and bacterial endosymbionts and gene duplication [13]. On the other hand, the ancestral NCLDV could have been a huge virus or even a cellular organism that evolved through genome regression [9,14-16].

A well-studied NCLDV is *Ectocarpus siliculosus* virus-1 (EsV-1), a phaeovirus that infects a small marine filamentous alga, *Ectocarpus siliculosus*. Phaeoviruses belong to the Phycodnavirus family. They possess icosahedral morphologies with internal lipid membranes and large double-stranded DNA genomes [17]. EsV-1 and other phaeoviruses only infect free-swimming, wall-less gametes or spores. One copy of the DNA is integrated into the cellular genome and then transmitted via mitosis through all cell generations of the developing host [18-20]. The viral genome remains latent in vegetative cells and is expressed in cells of the reproductive algal organs, sporangia and gametangia only when stimulated by, for example, changes in light composition and temperature [17,21]. Infected algae show no apparent growth or developmental defects other than partial or total inhibition of reproduction and phaeoviruses are pandemic in several brown algal species examined [17]. In addition to its lysogenic life cycle, the genome structure is the major feature which distinguishes EsV-1 from other NCLDVs. The genome is circular, but sequencing the genome resulted in a linear molecule of 335,593 bp ending in inverted repeats. Such a molecule might be able to assemble and form a circular genome [22,23]. It also contains numerous single-stranded regions randomly distributed over the genome whose functions remain unknown [24].

Another characteristic of the genome is its low gene density compared to other NCLDV genomes. The 231 genes occupy only 70 % of EsV-1 genome; these were assembled in islands of densely packed genes that are separated by large regions of DNA repeats and noncoding sequences [23]. In fact, the EsV-1 genome resembles a small eukaryotic chromosome more than a typical viral genome. A comparative analysis of the EsV-1 genome with the genome of two other phycodnaviruses indicated that they

could have evolved by loss of genes from a common ancestor, possibly an endosymbiotic organism, thereby supporting the theory of genome regression [25].

To elucidate how the EsV-1 genome is replicated, we first tried to determine where the viral genome is integrated into the host genome. We screened a cosmid library of *Ectocarpus* algae infected by EsV-1 using labelled EsV-1 DNA as a probe. Sequencing and analysis showed that the host chromosomal DNA was interspersed with single copies of small fragments of viral DNA. However, the fragments diverged in their DNA sequence from the corresponding EsV-1 DNA sequences. In the vicinity of these segments, we found other viral genes and pseudogenes, some of which are present only in the genome of the mimivirus. This finding indicates that the *Ectocarpus* genome contains remnants of the genome of either one giant dsDNA virus or one unicellular organism; this organism could be the ancestor of the contemporary NCLDVs.

Furthermore, this hypothesis is supported by previous data, indicating that the viral integrated fragments might be reassembled to constitute the EsV-1 genome. A putative protein coded by the EsV-1 and algal genome might be a key enzyme of this mechanism due to its similarity to tyrosine recombinases.

## Results

To determine where the viral genome is integrated in the host chromosome, we screened a genomic library of *Ectocarpus siliculosus* sporophyte NZVicZ14 infected by EsV-1. The strain NZVicZ14 was created by mating a male gamete from the strain NZ88 15d2 m containing the EsV-1 genome with a non-infected female gamete from the strain Vic88 12-15 f [26]. The DNA used for constructing the NZVicZ14 library was isolated from filaments prior to the appearance of symptoms and EsV-1 virions in order to limit the number of positive clones containing viral DNA from EsV-1 particles. The library was screened with radio-labeled EsV-1-DNA that had been digested with the DNA restriction enzyme *Sau3A*. The screening isolated 200 positive clones whose cloned fragments have an average size of 35 kb. To distinguish clones containing EsV-1 DNA linked to algal DNA from clones containing only EsV-1 DNA, the isolated positive clones were screened with labelled algal DNA from Vic88 12-15 f female gametophytes.

Surprisingly, all the clones were positive, indicating that they also have algal DNA. Consequently, no clone possessing only EsV-1 DNA was isolated. This result may be due either to the absence of viral particles in the algae or to the single-stranded DNA regions of the EsV-1 genome: these regions make the viral DNA fragile and therefore

might render large DNA fragments of EsV-1 in the cosmid vector unclonable [22].

The terminal regions of the EsV-1 genome are thought to be recognized by a putative protelomerase coded by the EsV-1 genome which could in turn linearize the EsV-1 genome and form telomeres [27]. So far, however, experiments to investigate this possibility have not succeeded. To investigate the hypothesis that the terminal repeats might also be the sequences involved in the integration of the viral DNA into the host genome, a second screen was performed using the probes A and A' corresponding to the terminal regions of the viral genome (Fig. 1). Of the 200 positive clones previously isolated, only four overlapping clones hybridized to both probes. Therefore, other clones have viral DNA that is not related to the terminal region. The DNA fragments of the positive clones can be assembled to form a 51-kb genomic region; such a region has been almost entirely sequenced and analyzed (Table 1 and Fig. 1, region A). The genomic region contains a ca. 5430 bp fragment that is 98 % identical to a segment overlapping the terminal region of the EsV-1 genome (Table 1, Fig. 1). The region of identity of the viral fragment and the EsV-1 genome is located just upstream of the promoter region of ORF 1 and ends approximately in the middle of ORF 231 (Fig. 1). The protein product of ORF 231 has no counterpart in the protein data banks [23]. Moreover, a recent blast search of the region indicated that ORF 231 is probably not a gene, since pieces of another ORF situated in ORF 231 code for remnants of a transposase which is related to those coded by the EsV-1 genome (Fig. 1) [23].

The integrated viral fragment contains the same DNA inverted repeats in the same orientation as that which characterizes the EsV-1 genome. Moreover, region A contains additional similar repeats at one extremity (Fig. 1). Apparently, the terminal DNA repeats of EsV-1 genome are longer than previously reported [23] (unpublished results). The discrepancy in the DNA sequence between the proviral and EsV-1 DNA sequences is due to small mutations, deletions or insertions, indicating that the integrated fragments have either accumulated mutations

during the evolution or come from an *Ectocarpus* virus other than EsV-1.

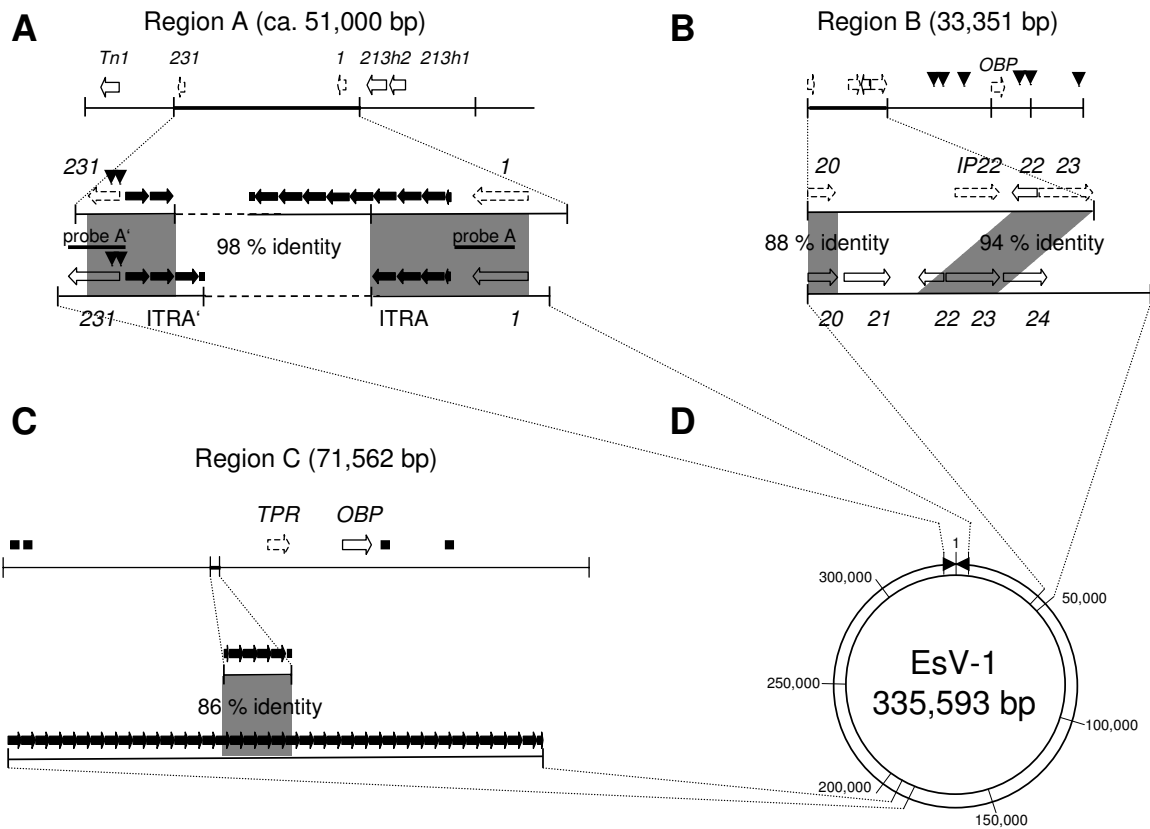
Several genes and remnants of genes were found in the vicinity of the viral fragments. Two homologues of ORF 213 of EsV-1 are present in tandem, upstream of ORF 1 (213h1 and 213h2, Fig. 1). ORF 213 is capable of encoding a large enzyme; this enzyme might be involved in DNA integration and recombination (Fig. 2). Protein 213 possesses at its C-terminus a tyrosine-recombinase domain resembling those found in the members of the integrase family whose hallmark is an invariant pattern of four amino acids (Arg, His, Arg, Tyr) forming the catalytic site [28]. ORF 213h1 and 213h2 do not have any similarity in their DNA sequences with ORF 213, indicating that ORF 213h1 and 213h2 are either algal genes or viral genes that NZVic14 horizontally acquired from another virus. The second copy of the gene is truncated at its C terminus resulting in the absence of a part of the tyrosine recombinase domain (Fig. 1 and 2). A homologue of ORF 213 was also found in the genome of another phaeovirus, the *Feldmannia irregularis* virus (FirrV-1), signifying that protein 213 might be essential for the life cycle of the phaeoviruses [25].

A putative transposase, Tn1, which shares identity with eukaryotic transposases of the Fot 1 family, is also present. It is predicted to contain a putative intron with 5'-AG/GUGAGG and 3'UGCAG/GU splice site sequences as well as a branch point UCAC. The genes of EsV-1 genome do not contain introns, whereas these are common in the algal genes. This therefore supports the contention that region A is composed of viral DNA framed by algal DNA.

In order to know whether other clones contain viral DNA, several DNA inserts were partially sequenced and analyzed. Viral DNA identical to EsV-1 DNA was missing. Of these sequenced inserts, one contains viral DNA at one extremity (Table 1; Fig. 1, region B). This clone, which has been sequenced to completion and analyzed, contains two fragments which have 88 and 94 % identity to EsV-1 DNA (Fig. 1 and Table 2). The fragments possess pseudo- and homologues of ORF 20, 22 and 23 from EsV-1 (Fig.

**Table 1: Comparison of the viral integrated fragments of regions A, B, C vs the EsV-1 genome.**

<i>Ectocarpus</i> genomic region	G + C of the genomic region (%)	Position of the viral integrated fragments	G + C of the viral fragments (%)	Position of the similar fragment in the EsV-1 genome	Identity
A	52.30	7036 – 8941 11748 – 15413	48.22 50.8	332959 – 3720	5431/5414 (98 %)
B	51.3	7 – 934 6586 – 9708	52.5 51.5	37542 – 38469 40867 – 43989	833/938 (88%) 2978/3153 (94%)
C	53.3	25376 – 26459	51.75	190061 – 188934	989/1144 (86%)



**Figure 1**  
**Genetic organization and comparative genomic analysis of three *Ectocarpus* algal genomic regions.** A, B, and C, maps of the algal genomic and portions of the EsV-1 genome. Gray regions connect homologous genomic regions. Dashed line, unknown DNA sequence. Arrow, putative ORF; broken arrow, pseudo-gene; black arrow, DNA repeats; black square, remnant of ankyrin repeats; arrowhead, relic of transposase; Tn, transposase; numbers indicate ORFs or homologues of the EsV-1 genome; 213h1 and 213h2, homologues of EsV-1 ORF 213; OBP, origin binding protein; IP22, Isoleucine/leucine proline repeat; TPR, tetratricopeptide-repeat. D, Circular map of the EsV-1 genome. Triangles, the inverted terminal repeats, ITRA and ITRA'. Numbers, nucleotide coordinates.

1). They are arranged as in the EsV-1 genome but diverge at the DNA level. Moreover, region B has a pseudogene whose translated product contains FNIP repeats. The pseudogene is situated between ORF 20 and ORF 22 instead of ORF 21 (Fig. 1). Interestingly, the EsV-1 genome does not contain FNIP repeats; in contrast, the mimivirus genome codes for at least 8 proteins containing this class of repeat [9,29]. Remarkably, the region contains another pseudogene coding for an origin binding protein (OBP); this OBP has its best identity with the MIMIR1 protein, one of the two putative OBPs of the giant mimivirus (Fig. 3 and Table 2). Although the identity is low, the UL-9 helicase domain with its ATP-binding site and DEAD site is still recognizable (Fig. 3). The OBP is also coded by the genome of the Herpes virus and Asfarvirus but is absent in the EsV-1 genome. OBP protein plays a major role in the replication of viral DNA [30]. In

addition, region B contains relics of transposase as does region A (Fig. 1B).

The presence of an OBP-like gene immediately raises the question whether other mimiviral-like genes are present elsewhere in the NZVicV14 *Ectocarpus* genome. Other DNA inserts were sequenced and analyzed. A genomic region of 70 kb was assembled with fragments of positive clones (region C, Fig. 1C). Region C contains a small viral DNA insert having a few repeats that are almost identical to those present in region D of the EsV-1 genome (Fig. 1 and Table 1). Surprisingly, it also has a complete ORF, which is capable of encoding a large OBP. This putative OBP shares identity with MIMIR8, the second and largest OBP coded by the genome of the mimivirus (Fig. 3). Like its MIMIR8 and herpes viral counterparts, this OBP is made of the N-terminal domain of the archaeo-eukaryotic

EsV-1-213	MASQ--ATKRLKLSSEA--GHTTLRAGFDPDVAITSPRTKVTYKSPVTLKRVFERPTE--SQVKVYF--HARN-HSGVFPDRLS	77
213h-2	MASSSRDITKRAKLS----PITTLNDINFNWVDAE--KTHTKMTFRCPVTIDVVFSAPTPAQVQMFYESRI--GSSGKPFGLP	77
213h-1	MASS--RANKRAKVSSSAKEPVTTLDNKAFDEKDVAESKTHTKMTFRCPVTIDNVF--IAPTAIQVKVYFESRS--SKGEPFDHLP	80
FirrV-1-B4	-----MRAQQTITPFSLESVFERGELHDEADERS-----FVHHRQQR	37
EsV-1-213	DAEKLITWTESLHDNSCLMGYLVYSQQLPCAAENRVKIKSGSAHWNELN--VSTDRARLEORRAAMDRLRRNGSTLSAFYGLP	157
213h2	EDPILAWTESFHDNKTLMGFIVYSQQLP--KLLQRIKLNAAIYHWGHLDPASEKGRKLEORRSADDRLKRNGETP--SVFRGLP	159
213h1	EGEKLAWTESFHDNKTLMGFIVYVQLP--KLLQRIKLNAAIYHWAHLDNSSEKGRKLEORRNAVDRLKRNGETR--SVFRGLP	162
FirrV-1-B4	HRSEEEARRAVALNRF--L--GFLVYVQVPLSVLNGRLKLRQPEYFQVLD-----PNSKQHEERKEGLRWLQMAQASEVFD--L	112
EsV-1-213	YSVDMITKNVLEYMNIIDF--QSDLRHRFFLGRFAMTSSSEHGKMNASSVY--TSCSKALSMLYF--IVVILGRPCCLLESVILPLSVTE	239
213h2	HRVDTCANILAFMKMDVDEEDERLRFNRF--SLVAFERGQVGS--SSVSSCSMALSLWFA--TTVILARPCCLLESVILPLSVTE	241
213h1	YIVDTSANILAFMKMDVEEEDERFRFFIKREFSMIF--SKRGVSS--SSVY--TSCSMALSLWFA--TVLARPCCLLESVILPLSVTE	244
FirrV-1-B4	TGTTSPSLIDFVLRISVSRPR--CEDLFLKADFRRSKR-----ASAYSVCAATLSHLWVTTVILGRPCCLLESVILPLSVTE	187
EsV-1-213	RSVLEQITWGCPCGTHLDGPI--K--FQTNWREVFRAIGTPHRSINIVCLERAIEESYIGGKHVADCFALYNATRSAPGD--AKTIAGL	321
213h2	RKMMEQRF--RCPPECFDGP--IKSFT--TNWQDVFAIGTCHRNIDVYTLLEKACENK--YIEGVHVADVYAF--FRATMTAPGDMNSIAAL	323
213h1	RRAMEQRF--RCPPECFDGP--IQSFQTNWQDVFAI--DTHHRNIN--YTLLEKACEHKYIKGVH--ADVAVFRATMTAPDDMNSIT--AL	326
FirrV-1-B4	YKSL--SMSEF--PN-----SLFVRS--PVDWGS--L--CVPSG--YRHE--ECV--GLLELWAKESH--R--ELF--FCVYTKYK--LKCT--SRD--V--NADCTF	263
EsV-1-213	ANHYR--LNSK--IGVESGAVVQVLDVLR--K--PMEAVGKIDG--SVLCAIT--SYG--RSTLDVQARCLGL--QAGEHSLF--DCSVSNKLEIT	403
213h2	VQSYK--EHSKHG--VESGAVVGI--DVIDRDKPVEAIGKIDGGVLC--IF--ITYGERACAEV--RAAC--KLESGS--LFD--D--SVSNKLEIM	404
213h1	VRSYIQ--AKH--G--VESGAVVVIDVIRDKSVEAIGKIDGGVLC--IF--PAYGERACLK--VQ--ECS--GLEGG--SLF--D--SVSNKLEIT	407
FirrV-1-B4	SQ--MVV--VPCYD--V--VFKNN--V--EGRV--RH-----F--S--L--L--C--G--K--F--H--E--L	302
EsV-1-213	NHADIALW--KARK--RLK--V--L--K--V--N--D--S--L--D--P--A--M--N--K--K--I--S--E--G--K--M--L--D--P--C--D--P--A--S--E--R--M--H--A--M--L--Q--S--I--R--Q--V--L--P--L--K--T--I--G--E--A--L--G--V--S--M--D--H--P--S--A	485
213h2	AHFDC--LW--KARK--K--K--V--E--R--V--A--A--N--L--D--P--A--G--M--N--K--K--I--E--G--K--M--L--D--P--T--D--P--K--S--I--A--L--M--H--A--M--L--K--S--I--R--Q--V--K--P--L--K--N--M--I--G--T--A--L--G--A--S--L--E--Y--P--T--A	486
213h1	AHFDC--LW--KARK--K--K--K--V--E--R--V--A--A--N--D--P--A--G--M--N--K--K--I--E--G--K--M--L--D--P--T--D--P--K--S--I--D--L--M--H--M--L--O--T--I--S--G--V--W--K--P--L--K--M--I--G--E--A--L--G--A--S--L--E--Y--P--A	489
FirrV-1-B4	VVYHEV--H--Q--V--R--R--N--T--E--R--F--L--Q--V--Q--K--S--L--D--P--A--G--T--Q--K--R--K--M--L--D--R--V--E--S--N--V--T--A--V--K--M--T--H--V--M--A--C--K--T--W--K--Y--E--K--V--R--D--S--I--A--E--A--E--T--E	384
EsV-1-213	FLRISDEE--A--H--R--L--Y--E--H--A--S--T--K--G--M--V--Y--A--D--I--A--N--L--N--T--L--L--V--L--S--N--S--F--Q--R--S--Q--V--L--R--E--A--T--I--N--E--F--G--L--V--P--D--G--T--E--Y--R--O--I--F--K--D--R--A--F--K--T--A--G----A--S--S--G--S	565
213h2	FLRISDEE--E--G--K--L--Y--K--H--L--V--A--G--V--Y--N--D--I--A--N--L--C--T--L--L--L--S--F--S--F--Q--R--S--Q--V--L--R--E--S--T--V--D--E--F--V--L--V--S--G--G--T--H--Y--K--E--S--F--K--G--R--R--F--K--T--A--S--S--G--G--A--S--S--A	568
213h1	FLRITQ--E--E--G--E--G--L--H--K--H--V--L--T--A--G--V--T--Y--D--V--A--N--L--C--T--L--L--L--S--F--S--F--Q--R--S--Q--V--L--R--E--A--T--V--D--E--F--V--L--V--D--A--T--H--Y--K--L--S--F--K--N--R--R--F--K--T--A--S--S--G--G--T--S--S--A	571
FirrV-1-B4	AT--V--C--R--E--F--L--K--H--R--A--G--E--F--C--A--F--S--M--H--E--V--A--D--V--C--F--L--F--L--T--C--F--H--R--S--Q--V--R--D--S--I--V--S--E--V--V--S--R--T--G--P--G--Y--M--L--S--L--S--R--A--T--K--T--S--G--T--D--C--N--G--V	463
<i>S. aureus</i>	N--R--D--R--V--L--L--L--Y--A--T--G--I--R--V--S--E--L--V--N--T--K--K--Q--I--D--E--Y--A--N--G--V	164
<i>B. Subtilis</i>	G--R--D--Q--A--L--L--L--L--Y--A--T--G--M--R--V--S--E--C--C--S--I--T--I--N--D--V--D--L--F--M--D--T--V	168
<i>H. influenzae</i>	D--H--R--D--R--A--L--L--L--L--Y--S--S--G--L--R--L--S--E--L--O--G--D--I--N--S--I--N--T--R--V--E--V	164
<i>P. multocida</i>	D--H--R--D--R--A--L--L--L--L--Y--S--S--G--L--R--L--S--E--L--O--S--L--N--I--N--S--I--N--T--R--V--E--V	164
<i>P. aeruginosa</i>	G--R--D--R--L--M--L--E--V--L--Y--A--C--G--L--R--V--S--E--L--V--G--T--L--E--Q--V--N--L--R--Q--G--V--V	168
EsV-1-213	PPVSHFNLS--P--G--O--S--M--V--H--F--I--A--V--V--G--H--R--F--C--N--A--D--M--K--D--K--R--K--L--L--N--S--K--G--R--G--C--V--O--N--D--I--N--S--P--F--K--K--I--G--A--H--W--L--G--L--P--N--F--C--V--H--T--C--R--T--F--W--A--T--A	646
213h2	PPVSHFNLS--P--D--O--S--M--T--K--F--I--A--F--I--G--H--R--F--C--G--L--K--N--D--G--N--G--R--L--F--V--N--S--K--G--O--S--W--T--K--D--V--S--S--R--F--K--R--I--G--M--O--W--L--G--I--O--N--F--G--P--H--V--C--R--S--F--W--S--T--H	650
213h1	PPVSHFNLS--P--D--O--S--M--T--K--F--I--A--I--V--G--H--R--F--C--G--I--L--C--L--N--E--A--R--R--L--E--V--N--T--K--G--D--T--R--E--N--T--R--Q--S--F--M--O--I--L--V--Q--F--K--O--K--T--K--S--T--M--O--M--N--T--K--N--A	653
FirrV-1-B4	APVREF--E--L--S--L--S--O--S--M--V--H--F--I--K--V--F--G--S--R--P-----S--D--T--L--L--V--N--E--R--E--N--T--M--T--O--E--N--L--C--M--R--F--R--S--M--G--K--E--Y--L--G--I--S--N--L--S--P--H--A--M--R--T--F--A--S--H	537
<i>S. aureus</i>	G--V--S--E--I--H--P--H--K--L--R--H--T--E--A--T--H	247
<i>B. Subtilis</i>	S--T--L--H--I--H--P--H--M--L--R--H--T--E--A--T--H	255
<i>H. influenzae</i>	L--N--S--H--L--N--P--H--K--L--R--H--S--P--A--T--H	246
<i>P. multocida</i>	L--S--S--H--L--N--P--H--K--L--R--H--S--P--A--T--H	246
<i>P. aeruginosa</i>	I--G--S--I--S--P--H--T--E--R--H--A--T--H	252
EsV-1-213	ALNSGQVDF--E--S--N--I--E--D--F--C--S--F--L--Q--V--S--S--T--L--R--S--S--Y--M--S--A--A--G--N--S--A--A--H--T--I--G--H--E--V--L--G--S--V--V--T--S--A--T--S--C-----E--T--T--E--O--C--D--A--P--O--C--E--K--L	718
213h2	ALNSGQV--S--A--S--N--L--E--N--E--S--S--F--L--Q--V--S--S--T--L--R--S--Y--M--S--A--A--G--N--S--A--A--H--T--V--G--N--V--L--G--A--V--V--N--A--A--C--T--C-----E--T--T--E--K--G--I--R--P--Y--G--K--K--L	721
213h1	T--T--A--G--A--L--S--R--V--L--R--K--N--T--E--T--S--L--R--K--T--R	676
FirrV-1-B4	A--V--N--S--G--M--V--I--S--A--N--M--A--L--F--G--S--Y--I--Q--V--S--A--K--T--I--S--L--N--Y--S--A--S--T--E--T--E--A--H--V--L--G--R--V--L--G--D--V--M--N--C--G--S--S--N--S--S--S--S--T--P--A--A--V--V--Q--Y--K--A--S--N--R--P--N--G--K--V--I	619
<i>S. aureus</i>	L--L--N--Q--G-----A--D--L--R--T--V--Q--S--L--L--G--H--V--N--L--S--T--T--G--K--Y--T--H--V--S--N--Q--Q--I--R--K--V	284
<i>B. Subtilis</i>	L--L--N--E--G-----A--D--L--R--S--V--Q--E--L--L--G--H--S--N--L--S--T--T--Q--I--Y--T--H--V--S--K--E--M--R--N--T	292
<i>H. influenzae</i>	M--E--A--S-----S--D--L--R--A--V--Q--E--L--L--G--H--S--N--L--S--T--T--Q--I--Y--T--H--L--N--F--O--H--L--A--E--V	295
<i>P. multocida</i>	M--E--A--S-----S--D--L--R--A--V--Q--E--L--L--G--H--S--N--L--S--T--T--Q--I--Y--T--H--L--N--F--O--H--L--A--E--V	295
<i>P. aeruginosa</i>	L--L--N--H--G-----A--D--L--R--V--Q--M--L--L--G--H--S--D--L--S--T--T--Q--I--Y--T--H--L--A--R--A--R--I--Q--D--L	290
EsV-1-213	RRIRMEFVGEIRASLLK--FSGNAKLLFRDLVKKR--K--V--G--O--L--G--E--G--E--I--W--F--R--E--N--T--F--M--K--D--G--E--R--V--L--R--F--V--G--G--V--	786
213h2	SARRLEFAGEIRASLARF--G--N--G--R--R--L--F--R--E--M--L--O--K--R--N--S--O--L--G--D--I--E--T--W--F--R--E--N--T--F--F--C--E--G--D--E--R--L--E--Q--R--F--L--D--K--L--	789
FirrV-1-B4	ARRARDGRRREMVQ--S--W--E--R--F--S--G--N--A--R--A--C--E--S--L--V--N--R--R--T--S--A--L--G--D--D--K--W--E--Y--S--N--I--F--F--L--D--H--D--V--R--E--L--R--C--I--S--K--K--S--K--N	689

**Figure 2**  
**Alignment of the putative viral integrases of *Ectocarpus* (213h1 and 213h2), EsV-I and FirrV-I with segments of tyrosine recombinases from various bacteria.** Accession numbers: [AAK14627](#) (EsV-I), [AAR26879](#) (FirrV-I), [Q9KJF6](#) (*Staphylococcus aureus*), [NP\\_389496](#) (*Bacillus subtilis* str. 168), [P44818](#) (*Haemophilus influenzae*), [AAK03785](#) (*Pasteurella multocida* str. Pm70) and [Q9HXQ6](#) (*Pseudomonas aeruginosa*). Asteriks indicate the four invariant amino acids of the tyrosine recombinase domain (Arg, His, Arg, Tyr).

**Table 2: Putative and incomplete ORFs in the three *Ectocarpus* genomic regions.**

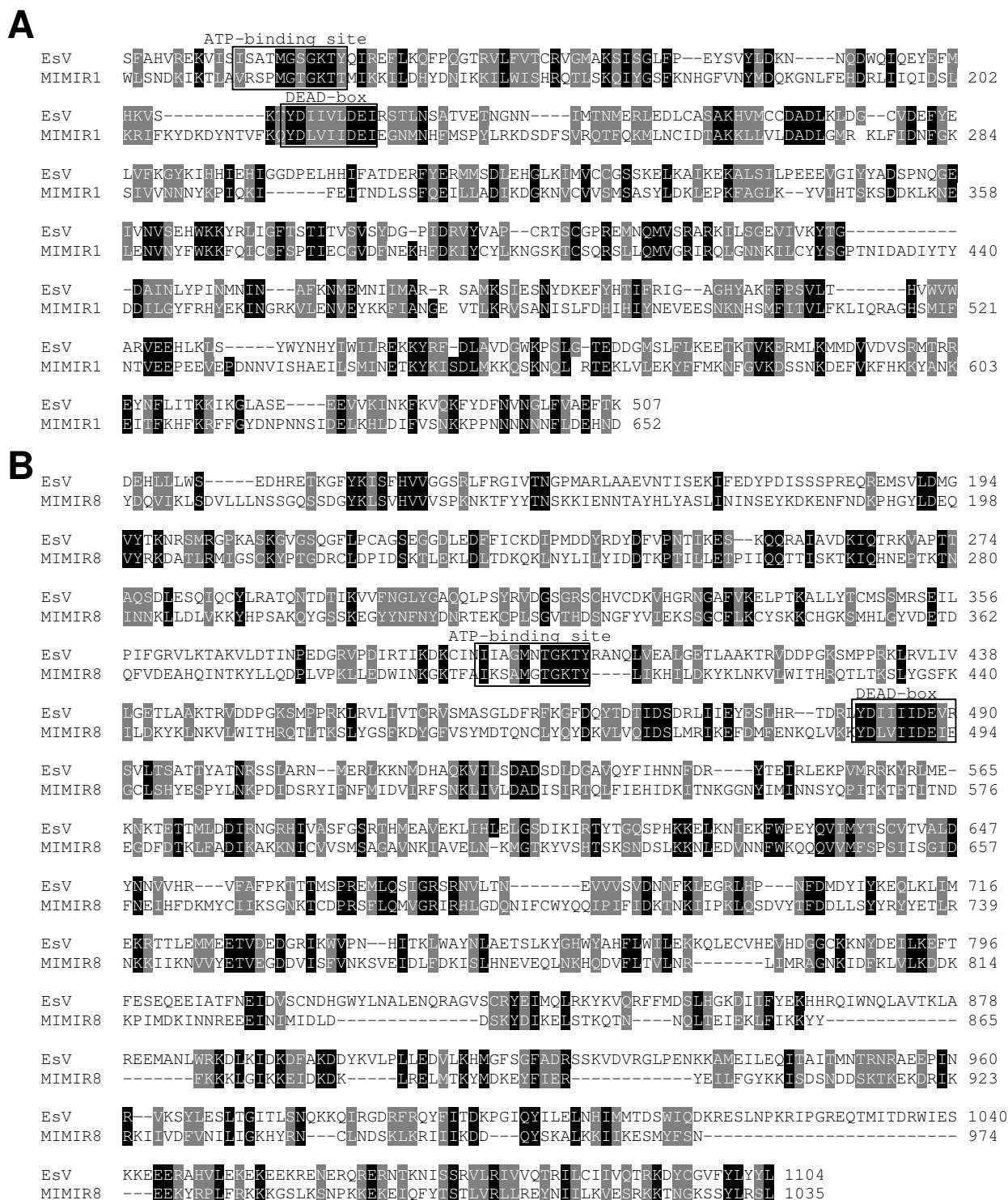
ORF	G+C content	Position	Size (aa)	Blast (E value)	Amino acid identity	Homolog(s) [accession number] <sup>a</sup>	Putative fonction/Features/comments <sup>b</sup>
213h1	54.24	21498 – 19471	675	0.0	350/615 (56%)	EsV-1-213 (length 786 aa) [ <a href="#">AAK14627</a> ]	Truncated homologue of EsV-1-213
				2e-35	138/551 (25%)	FirrV-1-B4 (length 689 aa) [ <a href="#">AAR26879</a> ]	
213h2	54.46	180467 – 15681	788	0.0	443/787 (56%)	EsV-1-213 (length 786 aa) [ <a href="#">AAK14627</a> ]	Integrase Phage integrase family (aa 490 – 690) [PF00589]
				1e-73	213/770 (27%)	FirrV-1-B4 (length 689 aa) [ <a href="#">AAR26879</a> ]	
				0.43	27/98 (27%)	<i>Bacillus subtilis</i> site-specific integrase/recombinase B4 (length 304 aa) [NP_389496]	
				0.95	38/85 (45%)	<i>Staphylococcus aureus</i> subsp. MRSA252 putative integrase/recombinase (length 298 aa) [YP_040639]	
TnI	56.95	6788 – 4524	541	1e-32	132/524 (25%)	<i>Aspergillus nidulans</i> FGSC A4 putative transposase (length 554 aa) [ <a href="#">EAA60308</a> ]	Transposase helix-turn-helix, Psq domain (aa 1 – 51) [PF05225] [PS50960],
				7e-32	110/384 (28%)	<i>Aspergillus awamori</i> transposase (length 555 aa) [ <a href="#">AAC49623</a> ]	
22h1	54.68	7793 – 6684	369	1e-139	250/289 (86%)	EsV-1-22 [NP_077507] (length 284 aa)	
OBP	47.12	41598 – 45086	1162	6e-11	160/730 (21%)	<i>Acanthamoeba polyphaga</i> Mimivirus helicase, similar to origin binding protein (length 1052 aa) [YP_142362]	Origin binding protein AAA (aa 386 – 562) [SM00382] [PF00004], Herpes_ori_bp (aa 401 – 930) [PF02399], ATP/GTP-binding site motif A (P-loop) (aa 394 – 401) [PS00017], Helicase domain (aa 381 – 720) [SM00490] [PF00271] [PSS1192] [PSS1194].
				2e-07	65/275 (23%)	<i>Acanthamoeba polyphaga</i> Mimivirus helicase, similar to origin binding protein (length 795 aa) [YP_142355]	

<sup>a</sup> Accession numbers are from the GenBank database.

<sup>b</sup> Pfam, prosite or Smart codes are in brackets.

primase (AEP) fused to the UL-9-like helicase domain containing an ATP-binding site and DEAD box [31]. However, the Zinc domain present in MIMIR8 is absent in *Ectocarpus* OBP. No additional ORF of viral origin was found except vestiges of genes coding for tetratricopeptide-repeats (TPR) and ankyrins (Fig. 1). Both kinds of repeats are the most frequent motif detected in putative ORFs of the mimivirus, while ankyrin-repeats were detected only in the EsV-1 genome [9,23]. Current sequencing of other clones indicates that the *Ectocarpus* genome contains several other genes of EsV-1 that could be assembled to form an apparently complete viral genome.

The variation in GC content in chromosomes is a good indicator of recent transposition and integration occurrences. The GC content, which is close to 52 % for the three genomic regions and the integrated viral fragments, shows no traces of a recent integration event (Table 1). Moreover, the GC content of the genomic regions is identical to that of the EsV-1 genome, which is much more than the 28 % GC content of the mimivirus [23,9]. In addition, the GC content of the OBP-like gene of the region C is 47 %, whereas it is only 28 % for the mimiviral gene (Table 2) [9]. Other reported ORFs have a similar GC content close to 52 % (Table 2). Taken together, these data suggest that the integration event of an EsV-like virus must have occurred a long time ago.



**Figure 3**  
**Alignment of segments of *Ectocarpus* OBPs with the mimiviral counterparts. A, OBP of region B with MIMIR1 (YP\_I42355). B; OBP of region C with MIMIR8 (YP\_I42362). The putative ATP binding site and DEAD region of helicases' superfamily are boxed.**

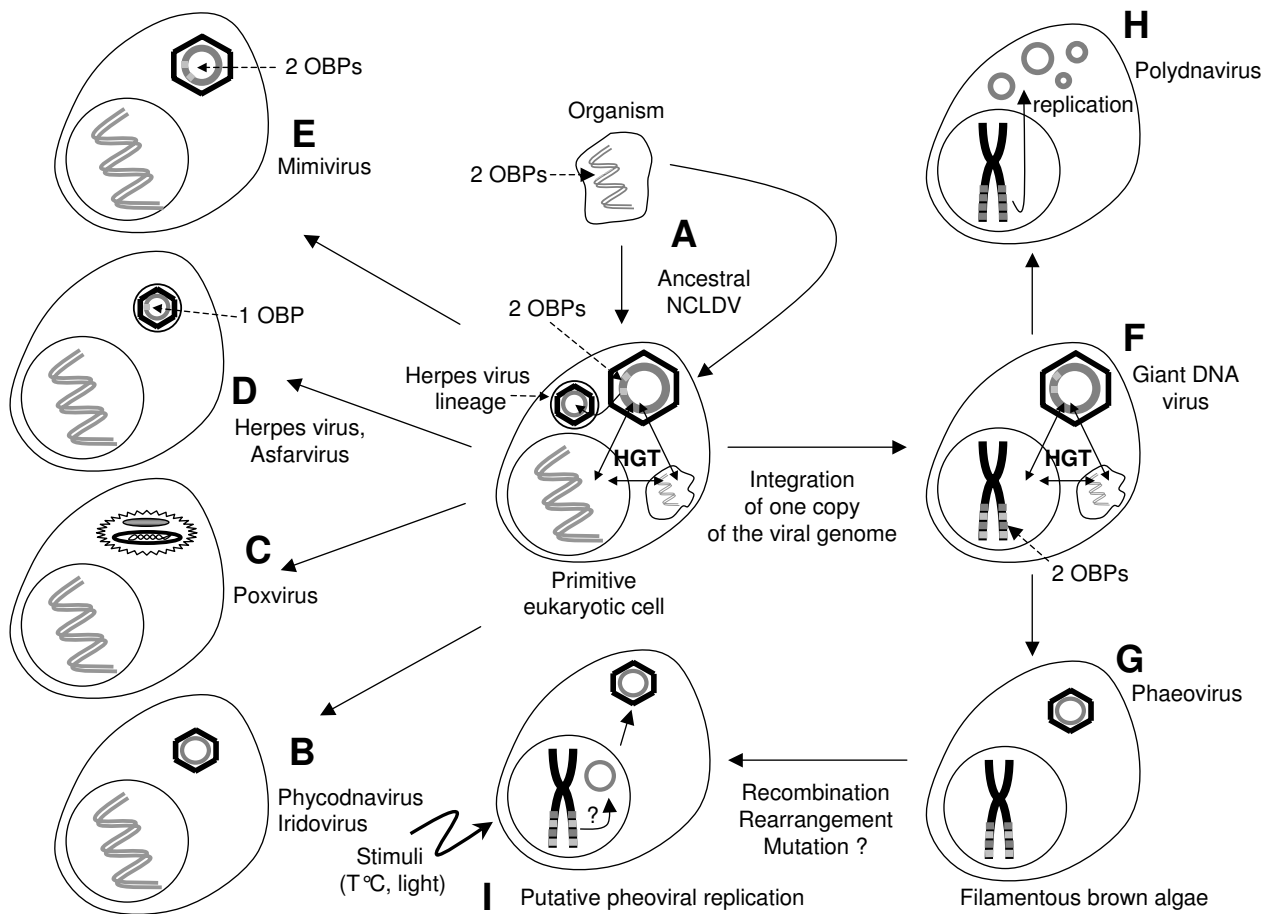
**Discussion**

**Ectocarpus' genome illustrates the evolution of NCLDV**

In this study, we showed that the genome of *Ectocarpus siliculosus* contains proviral fragments that share a high identity with regions of the genome of the EsV-1 and mimivirus. However, the integrated sequences are more similar in structure, gene organization and GC content and at the DNA and protein level to the EsV-1 genome than to the mimiviral genome.

The genome of *Feldmannia*, another filamentous brown alga which is not able to produce virions, also contains viral fragments [32]. In contrast to *Ectocarpus*, viral DNA sequences similar to mimiviral sequences have so far not been detected in the genome of *Feldmannia* algae.

Therefore, the presence of the viral sequences in the genome of *Feldmannia* and *Ectocarpus* raises the question of their origin. In the case of *Ectocarpus*, because screening isolated the one copy of the viral sequences, the most obvious explanation is that these viral sequences are remnants of one ancient viral infection. We believe that these integrated sequences represent traces of an ancestral NCLDV that could be either a giant virus or an even more complex organism (Fig. 4A). The occurrence of DNA sequences similar to mimiviral sequences among the EsV-1-like fragments indicates that these sequences might come from an organism having a large genome. Such an organism was probably at least as big as the mimivirus and may even have possessed the gene content of EsV-1 and mimivirus.



**Figure 4**

**Putative evolution of large DNA viruses.** A giant dsDNA virus or an endosymbiotic organism could be at the origin of the different classes of NCLDV (A). This organism would have evolved mainly through genome regression as shown by the loss of the OBP ORFs (B, C, D, E). In addition, the giant microbe has probably transferred its genome to the host genome (F) and evolved to give rise to the phaeoviruses (G) and polydnaviruses (H). It is likely that horizontal gene transfer (HGT) occurred between the different organisms present in the primitive eukaryote (A, F). For example, the herpesvirus OBP might have been acquired from the NCLDV ancestor. In the case of the phaeoviruses, the viral integrated fragments could serve as templates for the production of new virions through rearrangement, recombination and mutation (I).



Knowing that the OBP genes are either present or absent in the extant NCLDV, the presence of intact and disrupted genes coding for the two different mimiviral-like OBPs genes allows us to propose the following scenario for the evolution of the different classes of NCLDV (Fig. 4). The different NCLDVs evolved principally through genome reduction either of a big virus or of another organism, one that had at least two OBP genes (Fig. 4). In contrast to the phycodnaviruses, the iridoviruses and poxviruses, all of which have lost both OBP genes, the asfarviruses have lost one gene each; the mimivirus, on the other hand, has conserved both genes (Fig. 4B, C, D, E). Another class of viruses, herpes viruses, might also be derived from the ancestral NCLDV because they possess one OBP and the herpes viral primase probably derived from a NCLDV primase [31]. The herpes viruses would have lost one OBP gene during the evolution (Fig. 4D). However, investigations of the structure of the capsid protein of NCLDVs and herpes viruses indicate that these viral lineages probably originated independently [33]. The herpes viruses might have therefore acquired the primase and OBP genes from a member of the NCLDV family or the NCLDV ancestor (Fig. 4A) [31,12].

In addition, the genome of the ancestral NCLDV was probably integrated into the genome of a primitive eukaryotic cell that evolved to give rise to the filamentous brown algae. During its evolution, the integrated viral genome was presumably mutated by insertion and deletion as well as disrupted by successive integrations of transposons (Fig. 1A, B). The phaeoviruses evolved principally through genome regression, which led to the loss of both OBP genes supporting the hypothesis of genome regression [25]. However, surprisingly, *Ectocarpus* algae have retained the intact OBP gene, regardless of its usefulness for algae or viruses. The product of the OBP gene is therefore probably necessary for the replication of the viral DNA. In fact, the OBP gene seems to be present only in *Ectocarpus* algae that are capable of producing viral particles and to be absent in algae in which the virus is latent (not shown).

Analysis of several NCLDVs revealed the presence of genes evolutionary related to bacterial, archaeal and eukaryotic genes [9,34]. One hypothesis suggests that horizontal gene transfer (HGT) occurred among the NCLDV ancestor, the host and endosymbionts living in the cell (Fig. 4) [13]. However, it turns out that HGT is not sufficient to account for the mosaic structure and the huge size of certain viral genomes. For example, HGT was shown to participate only somewhat in the formation of the huge genome of the mimivirus, reinforcing the idea that the ancestral NCLDV was a microbe with a large genome [14].

Another class of viruses, the polydnviruses, could have evolved from the primitive cell that contained the ancestral NCLDV genome, given the similarities with the phaeoviruses. The polydnviridae are a family of mutualistic dsDNA viruses associated with parasitoid wasps that parasitize other insects [35]. The genome of the polydnviruses is present in two forms, as small DNA fragments within the genomes of wasps and as circular extra-chromosomal segments (Fig. 4H). As in EsV, the replication takes place exclusively in specialized regions of the wasp, the ovaries. The viral integrated segments are replicated, leading to the formation of the circular segments which are then injected into the prey along with the eggs. Proteins issued from the viral genes are responsible for suppressing the host's immune system and allowing the wasp's progeny to develop. In addition, although some integrated fragments are not present as circular segments after the replication, some products translated from these fragments are necessary to achieve the replication of the circular segments [36]. As mentioned above, the product of the OBP gene of region C might also be necessary for the EsV-1 genome to replicate itself. As in EsV, the transmission of the integrated segments follows Mendelian rules [37].

Even though polydnviruses do not have any gene in common with phaeoviruses, the genome shares several characteristics with the phaeoviral genome, such as many genes that code for proteins with ankyrin domains. Furthermore, the genome of polydnvirus possesses remnants of transposon and retrovirus-like elements, such as the viral integrated fragments [38,37]. A possible symbiosis has also been mentioned for *Ectocarpus* and EsV, in part because the growth of *Ectocarpus* has been shown to be unaffected by the presence of the virus [39]. Moreover, some protein products coded by the viral genome might confer some advantages on the algae: thaumatin, for example, is a pathogen-related protein involved in plant defenses [23].

#### **A new system for integration, recombination and mutation?**

The possible homology with polydnviruses lets us suppose that EsV also shares a similar replication system. We propose that the EsV-1 genome originates from these viral integrated sequences by means of an original recombination and rearrangement system that is triggered by environmental stimuli (Fig. 4I). Several facts support this hypothesis. First, the brown alga *Feldmannia* sp. is able to produce viruses having genomes with two different sizes and two variants of each, depending on the temperature at which the algae are cultivated [40]. Similarly, *Feldmannia irregularis* algae can produce viruses possessing numerous genomes varying from a few kb to 180 kb [25]. In the latter case, the composition of the viral genomes and the

identity of the genes sometimes differ greatly but the order of the genes remains the same. It seems unlikely that the host genome contains one copy of each viral genome. Thus, a system may exist which produces several different viral genomes using one or a few copies as template and integrated in the algal genome.

Second, the EsV-1 and FirrV-1 genomes encode a large putative integrase/ recombinase for which no counterpart exists in current protein databases. Only the Tyr-recombinase domain at the carboxy terminus of this enzyme is similar to phage integrases (Fig. 2). Therefore, this enzyme might be involved in the integration and/or recombination of the integrated viral fragments (Fig. 4). Moreover, the presence of two tandem copies of this enzyme accompanied by an ORF encoding a transposase and relics of another one strengthens the assumption that this enzyme is also involved in transposition.

However, the discrepancy between the integrated viral sequences and EsV-1 DNA raises questions. Such a discrepancy could be explained by the intervention of a mechanism that would edit the DNA sequences by rearrangement, recombination and mutation, leading to the formation of several different genomes. For instance, the viral recombination system could resemble the systems responsible for the diversity of the immune receptor genes [41].

The difference observed in the DNA sequence could result from one of two other factors. (i) Errors caused by the DNA polymerase during the synthesis of new viral DNA [42]; or (ii) the reparation of the viral DNA during or after replication as in *E. coli*. In the bacterium, the integrity of the bacterial DNA is restored after damage when it is single-stranded [43]. This could explain the presence of single-stranded DNA regions in the EsV-1 genome [24]

It can also be argued that these integrated fragments are ancient viral sequences that are degenerating, and the EsV-1 genome is present as an extra chromosome. However, no episomal structures were observed in the reproductive cells [19]. If this turns out to be the case, it would be interesting to know whether the viral integrated fragments are replicated along with the EsV-1 genome or genetic exchange occurs between the viral genome and its integrated replica.

## Conclusion

The huge genome of certain NCLDV is believed to be mainly due to HGT from the different hosts they might have infected during their evolution and to gene duplication. However, such an explanation is not sufficient to account, for example, for the enormous genome of the mimivirus. Here, we show that the genome of a multicel-

lular eukaryote has acquired genes from a giant DNA virus or a unicellular organism probably as big as a mimivirus but nevertheless more similar to EsV-1 at a DNA level. A comparison with the EsV-1 and other NCLDV genomes indicates that the ancestor probably lost genes during evolution, thus corroborating the genome accretion theory. Consequently, the virus should be seen not only as a gene robber but also as a major actor in shaping the genome of brown algae; it may act to shuffle genes between species. We know that EsV-1 can infect other, related brown algae [17]. Moreover, building on former results, we propose that phaeoviruses have probably developed an original replication system. Further studies will be necessary to confirm this model.

## Methods

### Alga and virus cultures

NZVicZ14 is an infected sporophyte producing EsV-1 particles as well as zoospores depending on the cultivation conditions of the algae. The cultivation of algae as well as the isolation and purification of viruses have been previously described (Lanka et al. 1993; Kapp et al. 1997).

### DNA extraction and purification

The purification of viral DNA has been previously described (Lanka et al. 1993).

Total algal DNA was isolated from 1 g wet filaments. The algal filaments were ground to powder. The powdered filaments were mixed in 10 ml extraction buffer (0.1 M Tris, 0.7 M NaCl, 0.1 M EDTA, pH 8, 2 % (w/v) CTAB, 0.1 % SDS, 3.5 mM DTEC, 1 %  $\beta$ -mercaptoethanol, 3% (w/v) PVPP) and shaken at room temperature for 1 hour. One volume (vol) of chloroform was then added to the cell lysate and centrifuged 10 min at 3,000 g. After centrifugation, 0.2  $\mu$ g/ $\mu$ l RNase A was added to the supernatant and incubated on ice for 30 min. The supernatant was extracted twice with 1 vol phenol/chloroform and 3 times with 1 vol chloroform. The DNA in the supernatant was precipitated in the presence of 0.7 vol of isopropanol. The precipitated DNA was pelleted by centrifugation, dried and resuspended in 300  $\mu$ l of 10 mM Tris, pH8.5.

### Genomic library

Standard methods for performing restriction digests, ligation reactions and plasmid isolations were used. A cosmid library was constructed with 50  $\mu$ g algal DNA using the pWEB cosmid cloning Kit (Epigene) according to the manufacturer's instructions.

### Screening

The library was screened by colony hybridization [44]. The algal genomic library was pre-screened using a probe that consisted of EsV-1 DNA, which was partially digested with *Sau3A*. Positive clones were subjected to a second

screening with the probes A and A' corresponding to the terminal regions ITRA and ITRA'. All the probes were labelled with <sup>32</sup>P dCTP using a NEBlot Kit (New England Biolab). After hybridization and washing, the filters were exposed to film (Kodak XAR) for 1 to three days. Positive clones were picked and plasmid DNA was extracted and purified for further analyses.

Probes A and A' were prepared by PCR using the following primers: probe A, A5, CCGCCTCCCCACCCACATTCA and A6, GTCCAACCCATCCTCTCG; probe A', A7, TGTGGCGAGGATGCTGTCTGAAT and A8, GTGTCGAGCGCGTATGTTGAAAT. Thermocycling conditions were 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, followed by a final 5-min extension at 72°C.

### Sequencing

A shotgun library of each positive cosmid and plasmid DNA was constructed. DNA was partially digested with *Sau3A*. The 1–5 kb DNA fragments were gel purified, ligated into the *Bam*HI site of CIP-treated plasmid pUC19 (Fermentas), and transformed into *E. coli* Max Efficiency Stbl2 (Invitrogen). DNA sequencing was carried out with the big dye kit (Perkin-Elmer) and sequences were analyzed on an ABI 377 sequencer. The shotgun sequences were assembled with the SeqManII Lasergene Software (DNASTAR Inc.) until a coverage of 10 was reached. Gaps between DNA segments were closed using PCR.

### Analysis of sequence data

The open reading frames (ORFs) were identified with the laser-gene biocomputing software package (DNASTAR Inc.). Homology searches were carried out with the blast program [45]. Protein motifs were searched against the SMART [46] and Pfam [47] databases. Sequence alignments were performed with the Megalign program (DNASTAR Inc.).

### Nucleotide sequence accession numbers

The DNA nucleotide sequences of the contigs have been deposited in GenBank under the following accession numbers: [EU254745](#), [EU254746](#) and [EU254747](#)

### Authors' contributions

ND conceived of the study, carried out the molecular genetic studies, provided the biological interpretation of the results, and finalized the manuscript. WB drafted the manuscript. All authors read and approved the final manuscript.

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