Epstein-Barr Virus BGLF4 Kinase Induces Disassembly of the Nuclear Lamina to Facilitate Virion Production

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Epstein-Barr Virus

- \( \gamma \)-herpesvirus
- Human herpesvirus 4 (HHV4)
- Enveloped virus
- Linear ds DNA, \(~180\) kb
- Tissue tropism: B cells, T cells and epithelial cells
Epstein-Barr Virus Life Cycle

Latency proteins:
- EBNAs
- LMPs

Immediate-early proteins
- Early proteins
- Late proteins

Latency Stage
Spontaneous activation
Stress (heat shock, UV)
TPA/Sodium butyrate
Anti-IgG

Lytic Stage
Epstein-Barr Virus Associated Diseases

Direct:
Infectious mononucleosis (IM)

Relative:
Hodgkin’s disease (HD)
Burkitt’s lymphoma (BL)
Post transplant lymphoproliferative disorder (PTLD)
Nasopharyngeal carcinoma (NPC)
EBV Lytic Cycle

Immediate-early genes
- Rta, Zta

Early genes
- BMRF1, BALF5, BALF2, BGLF4…etc

Late genes
- BFRF1, BFLF2…etc

Transactivation
DNA replication
Package, Release, membrane protein
EBV nucleocapsid

ONM             INM

NPC

38 nm

120 nm

EBV nucleocapsid
Nuclear Membrane

ONM
Outer membrane

INM
Inner membrane

NPC
Nuclear pore complex

The Cell: A Molecular Approach, 4e
Nuclear Lamina

- A electro-dense meshwork lining the INM (nucleoplasmic face)
- Composed by lamin A, B1, B2, C
- Provide structural support for nuclear membrane

Aebi et al., Nature. 1986
Nuclear Lamina

- Bind to many INM proteins (emerin, lamin B receptor)
- Bind to chromatin, DNA replication complex

Goldman et al., Genes Dev. 2002
Lamin

- Type-V intermediate filament
- A type: lamin A, C(\textit{lmnA})
  - nuclear stiffness
- B type: lamin B1, B2(\textit{lmnB1, B2})
  - fundamental structure of lamina
  - nuclear shape

Graphical representation of lamin A/C and lamin B with head, rod, and tail regions.
Lamin assembly

Lamin polypeptide

Dimer

Head-to-tail association of dimers

Polymer

Side-by-side association of polymers

Higher-order structure

The Cell: A Molecular Approach, 4e
Nuclear lamina disassembly in M phase

The author assume that BGLF4 mimic Cdc2 function.
**EBV BGLF4 kinase**

- EBV early gene during lytic cycle
- Ser/Thr protein kinase
- Belong to the conserved family of herpesviral UL kinases
- Substrate:
  - BGLF4, BMRF1, EBNA-2, EBNA-LP, Zta
  - EF-1δ, condensin, topoisomerase II, MCM4
- BGLF4 phosphorylates EF-1δ at Cdc2 target sites.

Kawaguchi, Y. et al. (2003). J Virol **77**:2359-68
Aims

- Determine how EBV BGLF4 kinase regulates the dynamics of the nuclear lamina.

- Explore the mechanism used by BGLF4 to regulate the egress of nucleocapsid.
BGLF4 induces nuclear lamina disassembly?!
BGLF4 interacts with lamin A/C and induces nuclear lamina disassembly

- **BGLF4** interacts with lamin A/C
- *Co-IP* (Co-immunoprecipitation assay)
- *IFA* (Immunofluorescence assay)
- *HeLa cells* (Confocal microscopy)
- Kinase dead mutant
- K102I

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**Images**:
- **Vector**
- **BGLF4**
- **K102I**

**Results**:
- Immunofluorescence assay
- Co-IP

**Assays**:
- Immunofluorescence assay
- Co-immunoprecipitation assay

**Protein Detection**:
- Lamin A
- Lamin C

**Western Blot (WB)**:
- α-Lamin A/C
- α-BGLF4

**Immunostaining**:
- Hoechst (nuclear stain)
BGLF4 phosphorylates lamin A in vitro

Immunoprecipitation (IP) kinase assay

HeLa cells

Immunoprecipitation (IP) kinase assay


Cdc2 (CDK1)
Ser-22,390,392 of lamin A are important for BGLF4-induced nuclear lamina disassembly

IFA (Confocal) HeLa cells
Ser-22,390,392 of lamin A are important for BGLF4-induced nuclear lamina disassembly

<table>
<thead>
<tr>
<th>Lamin A</th>
<th>Head</th>
<th>Rod</th>
<th>Tail</th>
<th>CaaX</th>
<th>BGLF4-induced nuclear lamina disassembly</th>
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Would nuclear lamina be disassembled by BGLF4 during EBV lytic cycle?
BGLF4 induces nuclear lamina disassembly during EBV reactivation.
BGLF4 induces nuclear lamina disassembly during EBV reactivation

Knockdown BGLF4!!!
Nuclear lamina can be disassembled by BGLF4 during EBV reactivation.

What are the effects of nuclear lamina disassembly on the EBV virion production?
Effects of nuclear lamina disassembly on the EBV virion production
Effects of nuclear lamina disassembly on the EBV virion production

EREV8 cells

Early protein

Late protein

Late protein
EBV late protein BFRF1, BFLF2

- EBV late gene
- Primary envelope proteins
- BFRF1 → cytoplasm
- BFLF2 → nuclear
Lamin A mutant reduce the expression of EBV late gene BFRF1/BFLF2

EREV8 cells
Lamin A mutant reduce virion production

PCR

EREV8 cells