

Opacity-associated (Opa) Protein Family

By Kathleen Nicholson

Opacity-associated (Opa) Proteins:

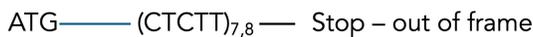
- Origin of the name: Colonies expressing Opa-family proteins exhibited opaque phenotypes when observed with oblique light (dissecting microscope), whereas colonies not expressing Opa proteins exhibited transparent colony phenotypes.
- There are at least 11 *opa* genes in *N. gonorrhoeae*, which produce 7-9 unique Opa proteins.
- *opa* genes undergo phase variation: pentameric pyrimidine repeat sequences $(CTCTT)_n$ are variable, wherein slipped-strand mispairing can result in in-frame or out-of-frame coding regions.
- There is selection for *opa* expression during human infection.
- Opa proteins are between 25 and 30 kDa in size.
- *opa* genes have hypervariable (HV_1 and HV_2) and semi-variable (SV) regions that encode different extracellular loop amino acid sequences.

opa Slipped-strand Synthesis

Gene layout:



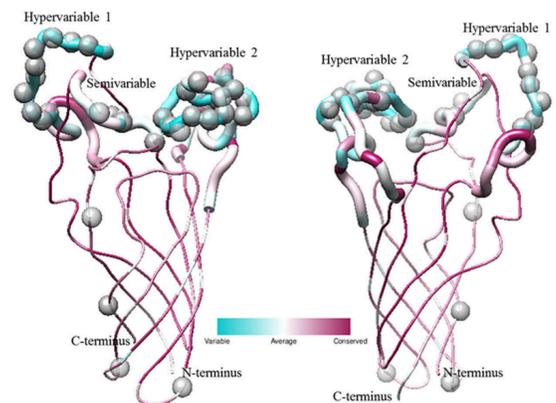
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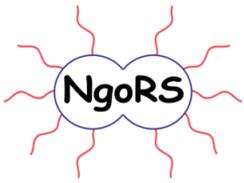
Full length product:



Proposed Opa Structure*



*Figure from: Wachter J, Hill S (2016) Positive selection pressure drives variation on the surface-exposed variable proteins of the pathogenic *Neisseria*. *PLoS One*. 11(8):e0161348.

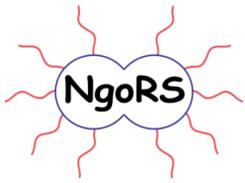


Opa Proteins in Pathogenesis:

- The surface-exposed hypervariable (HV₁ and HV₂) and semi-variable (SV) regions of Opa proteins confer specificity to different host receptors.
- Most Opa proteins interact with human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs, previously designated as CD66).
- Some Opa proteins bind heparan sulfate proteoglycans (HSPGs).

CEACAM ¹	Opa Interaction	Cell Line Expression	Transmembrane or GPI-linked
CEACAM1 (CD66a)	Yes	Epithelial cells, endothelial cells, monocytes, granulocytes, activated T and B cells, myeloid cells, natural killer cells	Transmembrane
CEACAM3 (CD66d)	Yes	Neutrophils and other granulocytes (eosinophils, basophils)	Transmembrane
CEACAM4	No	Primary human granulocytes	Transmembrane
CEACAM5 (CEA; CD66e)	Yes	Epithelial cells (gastrointestinal, nasopharynx, esophagus, lung, urogenital tract), Vascular endothelial cells, Monocytes	GPI-linked
CEACAM6 (CD66c)	Yes	Granulocytes, Myeloid cells in prostate and bone marrow, Epithelial cells (stomach, liver, colon, gallbladder, skin, tongue, esophagus, cervix)	GPI-linked
CEACAM8 (CD66b)	No	Neutrophils	GPI-linked

1 – Additional CEACAMs exist (e.g., CEACAM-7, -16, -18, -19, -20, and -21); however, their role in neisserial pathogenesis is unknown/not studied.

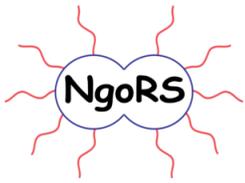


Opacity Protein Nomenclature

NGO Number based on genomic location in strain FA1090	Gene Name (historical)	Antibody ¹	Notes
NGO_0067 (NGO_00350 - Uniprot)	<i>opaA</i>	H.138	
NGO_0070 (NGO_11100 - Uniprot)	<i>opaB</i>	H.4	Lower apparent molecular weight than OpaD
NGO_0951 (NGO_08230 - Uniprot)	<i>opaF</i>	H.156	Duplication of <i>opaH</i>
NGO_1038/39 (NGO_05420 - Uniprot)	<i>opaC/J</i>	H.157	<i>opaJ</i> expression has not been detected; may be pseudogene
NGO_1076 (NGO_05620 - Uniprot)	<i>opaC/J</i>		<i>opaJ</i> expression has not been detected; may be pseudogene
NGO_1278 (NGO_06725 - Uniprot)	<i>opaK</i>	H.164	Slight difference in protein migration compared to OpaE
NGO_1464 (NGO_07725 - Uniprot)	<i>opal</i>		
NGO_1513	<i>opaD</i>	H.4	Higher apparent molecular weight than OpaB
NGO_1555 (NGO_08230 - Uniprot)	<i>opaH</i>	H.156*	
NGO_1862 (NGO_09965 - Uniprot)	<i>opaE</i>	H.164	Slight difference in protein migration compared to OpaK
NGO_2060.5 (NGO_11100 - Uniprot)	<i>opaG</i>	H.4*	Duplication of <i>opaB</i>

1 – 4B12 is a pan-Opa monoclonal antibody (mAb) that will detect all Opa proteins

* Presumed antibody, due to duplication



Nomenclature of Select Surface Antigens

Historical designations of select proteins and antigens:

Protein or Antigen	Designation	Prior Designation(s)
Porin	PorB ¹ PorB1.A and PorB1.B isotypes Various serovars/classes	Protein I (P.I, PI) Major Protein ² Major outer membrane protein (MOMP) Principal outer membrane protein (POMP)
Opacity-associated outer membrane protein (Opa)	Opa OpaA-I(J) serotypes	Protein II (P.II, PII, PII*) Protein 2 (P.2, P2, P2*) Minor protein (mP) Heat-modifiable proteins (hmP) Opacity-associated protein (OAP) Leukocyte-associated protein
Reduction-modifiable Protein (Rmp)	Rmp	Protein III (P.III, PIII) Protein 3 (P3) Protein 3,3* (P3,3*)
Lipooligosaccharide (LOS)	LOS	Lipopolysaccharide (LPS) Endotoxin
Pilin ³	Pilin (Pil) <i>pilE</i> gene	NA
H.8 antigen ⁴	H.8 antigen	NA ⁵

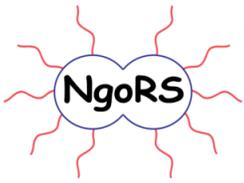
1 - Gonococcal nomenclature regarding porin is based on its homology to meningococcal PorB.

2 - Porin accounts for greater than 60% of the total weight of proteins present in the outer membrane of the gonococcus

3 - Pilin refers to each individual pilin subunit, which polymerize to form a pilus fiber.

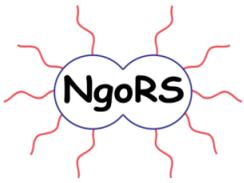
4 - The H.8 antigen comprises an AAEAP pentameric repeat found in the Lip (lipoprotein) and Laz (lipid-modified azurin) proteins.

5 - The H.8 epitope is named after the first monoclonal antibody hybridoma shown to recognize this surface epitope in pathogenic *Neisseria*



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