Cherwell Innovation Centre
Unit 77, Heyford Park
Upper Heyford
Oxfordshire, OX25 5HD
United Kingdom
Tel: +44 (0)1869 238 067
Fax: +44 (0)1869 238 001

Zaire ebola virus-like particles (ZEBOVLPs)

The Native Antigen Company's ZEBOVLPs were produced by transient transfection of 293 human embryonic kidney cells with vectors coding for Zaire ebola Nucleoprotein (NP), Glycoprotein (GP) and matrix protein (VP40). Virus-like particles were precipitated from raw culture supernatant, and were subjected to density gradient centrifugation and dialysis. Transmission electron microscopy was performed (Figure 1).

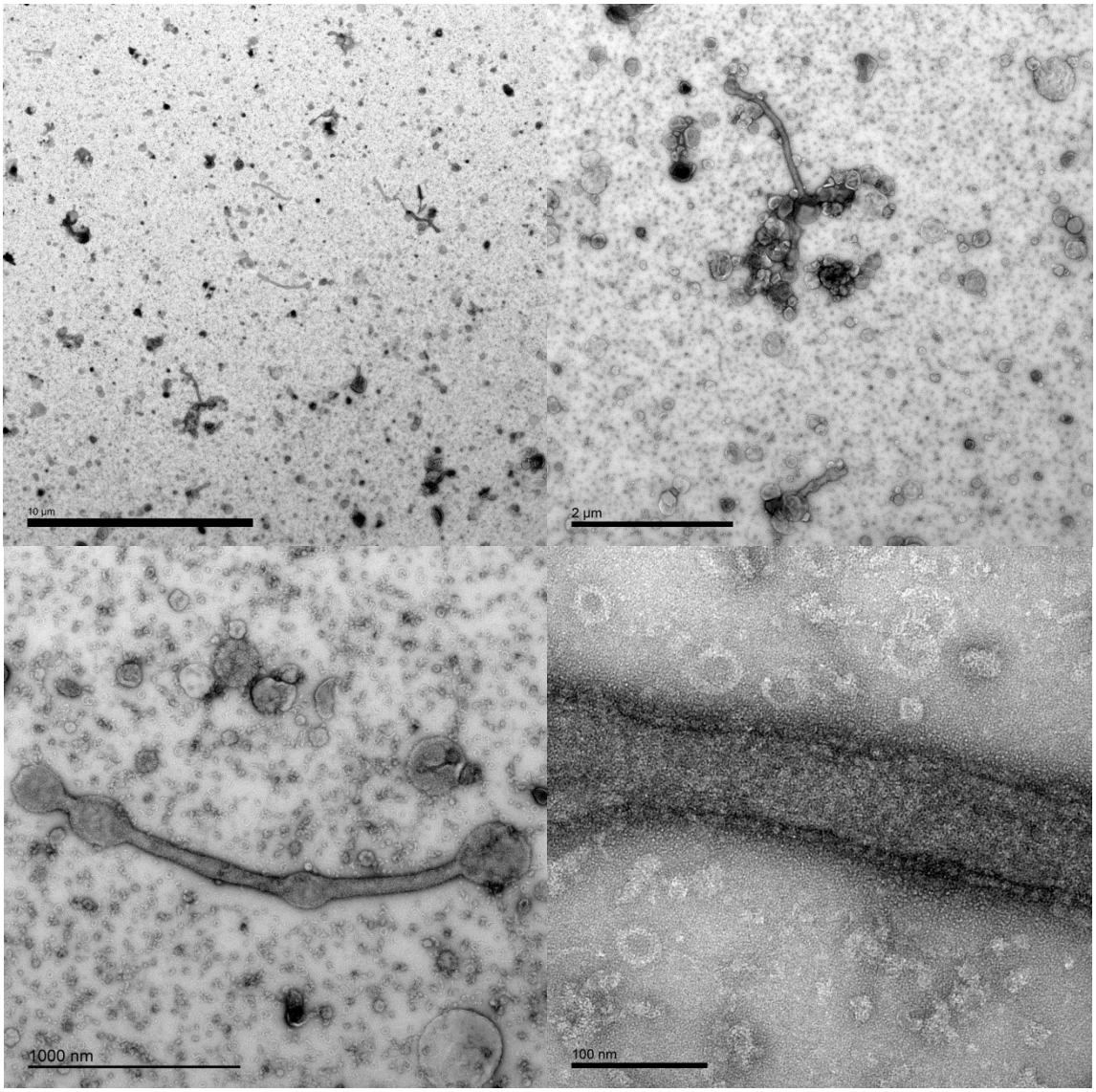

Figure 1: Transmission electron microscopy of Zaire ebola virus-like particles.

The micrographs confirm the presence of elongated structures (up to 2 um) and round vesicles (up to 200 nm in diameter) that are lined with pearl-like chains of electron dense material, probably ebola matrix protein VP40. Also, spiral structures (up to 40nm in diameter), possibly consisting of ebola NP and short cellular RNA species are visible, and may originate from ruptured VLPs during dialysis.

A Coomassie stained SDS-PAGE of above preparation shows a number of proteins. The six main bands (marked in Figure 2) were subjected to mass spectrometry (MS) fingerprinting.

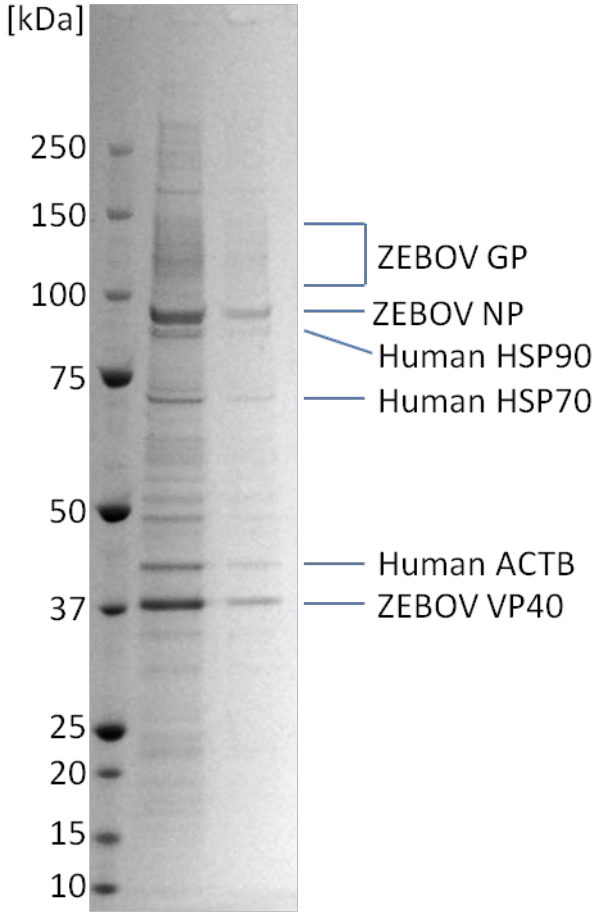

Figure 2: Coomassie stained SDS-PAGE. Markings correspond to bands analysed by MS fingerprinting, and protein names corresponding to MS fingerprinting results. ZEBOV: Zaire ebola virus, HSP90: Heat-shock protein 90, HSP70: Heat-shock protein 70, ACTB: beta-actin

As well as ebola virus VP40, GP and NP three human proteins were identified using MS fingerprinting: β-actin and the two heat-shock proteins HSP70 and HSP90.

Han and Harty could show co-packaging of β-actin into ebola VLPs by functional budding and protease protection assays when co-expressing VP40 and GP in 293T cells (Han Z).

It is known that human HSP70 and HSP90 interact with each other, and can be part of a larger chaperone complex (Pratt WB). Virus-yield reduction assays using HSP90 inhibitors showed marked reduction of ebola virus yields, and it was speculated that HSP90 might play a role in viral budding (Smith DR). In addition, HSP70 was found to be packaged into ebola virus particles by serial proteomics analysis and RNAi screen in a study performed by Spurgers and Bavari (Spurgers KB).

These studies show that at least these three identified human proteins could be essential parts of ebola VLPs.

Bibliography

Han Z, H. R. (n.d.). Packaging of actin into Ebola virus VLPs. *Virol J. 2005 Dec 20;2:92.*

Pratt WB, M. Y. (n.d.). Proposal for a role of the Hsp90/Hsp70-based chaperone machinery in making triage decisions when proteins undergo oxidative and toxic damage. *Exp Biol Med (Maywood). 2010 Mar;235(3):278-89. doi: 10.1258/ebm.2009.009250.*

Smith DR, M. S. (n.d.). Inhibition of heat-shock protein 90 reduces Ebola virus replication. *Antiviral Res. 2010 Aug;87(2):187-94. doi: 10.1016/j.antiviral.2010.04.015. Epub 2010 May 7.*

Spurgers KB, A. T. (n.d.). Identification of essential filovirion-associated host factors by serial proteomic analysis and RNAi screen. *Mol Cell Proteomics. 2010 Dec;9(12):2690-703. doi: 10.1074/mcp.M110.003418. Epub 2010 Aug 11.*