Chapter 2

Study of IAV NP-Clusterin interaction
4.2.1. Background

IAV infection in mammalian cells induces massive cell death, which is essential for successful virus replication [Ludwig et al., 2006]. Several viral proteins are known to contribute to IAV induced cell death. In this study we report for the first time IAV NP also induces cell death via intrinsic apoptosis program. It does so by interacting with host anti-apoptotic protein Clusterin (Clu). Clu is a highly conserved protein present in all types of body tissue [Trougakos et al., 2002]. It is translated as a 449 amino acid protein (60 kD) in intracellular form, which undergoes extensive post-translational modifications to form a secreted heterodimeric glycoprotein (80 kD) [Burkey et al., 1991]. The intracellular form of Clu (58-60 kD) is known to play regulatory roles in apoptosis, DNA repair, cell cycle regulation and cell signaling [Trougakos et al., 2002]. Clu plays an anti-apoptotic role by interacting with Bax and preventing its movement into the mitochondria [Zhang et al., 2005]. Keeping this function of Clu in mind, we investigated the role of IAV NP-Clu interaction in IAV-induced cell death. We observed that IAV NP can induce apoptosis when expressed from a plasmid in A549 cells, and that inhibition of NP expression in IAV infected cells leads to a reduction in IAV-induced cell death. Furthermore, NP-induced apoptosis was found to be mediated by its interaction with Clu as overexpression of Clu reduced apoptosis, while inhibition of Clu expression enhanced the apoptotic effect of IAV NP. Taken together, our data suggest that IAV NP targets human Clusterin protein and induces cell death in IAV-infected cells.
4.2.2. Results

4.2.2.1. Influenza A virus nucleoprotein interacts with Clusterin in human lung epithelial cells

In an attempt to identify novel cellular interacting partners of IAV NP, yeast two-hybrid screening of Human Lung cDNA library was conducted, using NP gene of A/Hatay/2004(H5N1) strain as bait [Sharma et al., 2011]. The sequence of cDNA insert in the positive clones was identified by sequencing and subsequent BLAST analysis. One of the positive interactors was identified as human Clusterin gene transcript variant 2. The NP-Clusterin interaction was verified by co-immunoprecipitation from A549 cells which were transfected with plasmids expressing Myc tagged NP gene of A/Hatay/2004(H5N1) IAV and human Clusterin gene. IAV NP could immunoprecipitate (Fig.1 A, lane 2, panel 2) the overexpressed Clu and vice-versa (Fig.1A, lane 2, panel 1).

Interaction between NP and Clu was verified by co-immunoprecipitation from A549 cells infected with A/Puerto Rico/8/1934(H1N1) IAV at 1 MOI for 24 hours. The endogenously expressed Clu co-immunoprecipitated with IAV NP (Fig.1B, lane 1, panel 2) and vice-versa (Fig.1B, lane 1, panel 1). The molecular weight of Clu protein which immunoprecipitated with IAV NP was 58-60 kD which corresponds to the intracellular form of Clu. This interaction was further confirmed in A549 cells infected with several seasonal and pandemic isolates of IAV. Human Clu protein co-immunoprecipitated with NP protein of all the IAV isolates tested in the experiment (Fig.2, panel 1). Furthermore, NP and Clu co-localized extensively in the perinuclear region in A549 cells infected with PR8 IAV at 5 MOI for 6 hours (Fig. 3A). Also Clu localization seemed to change from diffused nucleo-cytoplasmic in control cells to perinuclear in case of IAV infected cells (Fig. 3B).
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Fig. 1. IAV NP interacts with cellular Clusterin in human lung epithelial cells, transiently expressing NP or infected with Influenza A Virus

A. A549 cells were co-transfected with pcDNA3.1-NP + pCMV-Clu plasmids or control pcDNA3.1+pCMV plasmids and harvested 48 hours post-transfection. Immunoprecipitation was setup using anti-Myc tag antibody and anti-Clu antibody, followed by western blotting. Lane 2 of panel 1 shows co-IP of NP with Clu and lane 2 of panel 2 shows co-IP Clu with NP. Lane 1 of panel 1 and 2 represents control samples transfected with empty vector. Panels 3 and 4 shows expression levels of NP and Clu in cell lysates. Panel 5 shows loading control β Actin.

B. A549 cells were infected with PR8 IAV at 1 MOI and harvested 24 hours post-infection. IP was setup using NP-specific antibody and Clu-specific antibody. Lane 1 of panel 1 shows co-IP of NP with Clu and lane 1 of panel 2 shows co-IP Clu with NP. Lanes 2 of panel 1 and 2 represents control uninfected samples. Panels 3 and 4 shows expression levels of Clu and NP in cell lysates, as detected by western blotting. Panel 5 shows loading control β Actin.
Fig. 2. IAV NP-Clusterin interaction is conserved in several seasonal and pandemic strains of influenza A viruses

A549 cells were infected with different influenza A virus isolates, as indicated at 1 MOI. Cells were harvested 24 hours post-infection and IP was setup using NP specific antibody, followed by western blotting. Panel 1 shows co-IP of Clu with NP, panels 2, 3 and 4 show levels of Clu, NP and β-Actin in the cell lysates used for IP. Lane 1 of all panels show control uninfected samples.
Fig. 3. IAV NP and Clusterin co-localize primarily in perinuclear region of cells

A549 cells were infected with PR8 influenza A virus at 5 MOI for 6 hours, and cells were fixed and processed for immunostaining. NP was stained using anti-NP monoclonal primary antibody and Alexa 488 conjugated secondary antibody (Green). Clu was stained using Clu specific primary antibody and Alexa 594 conjugated secondary antibody (Red). Nuclei were stained with Hoechst stain. Panels are labeled for their respective staining. **A.** Shows PR8 IAV infected cells; Image region showing co-localization is indicated by arrows. **B.** Shows control uninfected cells.
4.2.2.2. Ectopic expression of IAV nucleoprotein induces cell death in human lung epithelial cells

Clusterin is an anti-apoptotic protein, thus we hypothesized that IAV NP may interfere with its cell survival function. To test this hypothesis, we examined the ability of IAV NP to induce cell death by transfecting A549 cells with either pEGFP-NP plasmid (expressing H5N1 IAV NP fused to GFP) or the control pEGFP plasmid. 48 hours post-transfection, cells were harvested, stained them with Annexin V PE and 7 AAD dyes and tested them by flow cytometry. Annexin V is a marker for early apoptosis whereas 7AAD is indicator of late apoptotic events [Vermes et al., 1995; Lecoeur et al., 2002]. GFP +ve populations were gated-out and analyzed for Annexin V or 7AAD staining. Data indicated that IAV NP induced \(\approx 3\) fold more cell death as compared to control treated cells (Fig. 4A, B). The degree of apoptosis depended on the expression level of NP as the apoptotic events increased by increasing IAV NP plasmid dose during transfection (Fig. 5A). Similar increase in apoptosis was observed by increasing the virus infection dose in A549 cells (Fig. 5B), however role of other viral factors in the same cannot be ruled out.
Fig.4. Ectopic expression of IAV NP induces apoptosis in human lung epithelial cells

A549 cells were transfected with pEGFP-NP plasmid or control pEGFP plasmid. 48 hours post-transfection cells were harvested and stained with Annexin V PE or 7AAD dye and subjected to flow cytometry. GFP +ve cells were gated out and among that population Annexin V+ve/-ve or 7AAD +ve/-ve population were analysed. The contour plots are representatives of at least three independent experiments. The select populations in the contour plots are gated and their percentage is mentioned in upper right corner of each plot. Data shown in the graphs represent means ± standard deviations of one representative experiment (n = 3). Statistical significance was determined using Student's t test. *, p<0.05; **, p<0.01; ***, p<0.001. A. Shows results of Annexin V PE staining. B Shows results of 7AAD staining.
Fig. 5. Induction of cell death by IAV NP in dose dependent manner

A. A549 cells were transfected with increasing amounts of pEGFP-NP or control pEGFP plasmid. 48 hours post-transfection cells were harvested and stained with Annexin V PE and subjected to Flow cytometry. Ratio of (GFP+AnnexinV PE) +ve population in pEGFP-NP over pEGFP transfected samples at respective doses is plotted on the graph. Amount of DNA used for transfection is mentioned at the bottom of each graph bar. B. A549 cells were infected with increasing doses of A/California/2009(H1N1) IAV strain. Cells were harvested 12 hours post-infection and stained with Annexin V PE and Annexin V +ve population was plotted on the graph. MOI of virus used for infection is mentioned at the bottom of each graph bar. Graphs represent mean ± standard deviation of each representative experiment (n = 3). All data shown are representatives of at least three independent experiments. Western blot from the same samples was done to detect NP levels and is shown below the graphs.
4.2.2.3. IAV NP-induced cell death is executed through the mitochondrial apoptotic pathway

Since Clu prevents the initiation of mitochondrial cell death pathway, we checked whether IAV NP-induced cell death is also mediated by the same pathway. Movement of Bax into mitochondria, release of Cytochrome c (Cyt c) from mitochondrial membrane and subsequent activation of Caspase 3 are hallmarks of the mitochondrial apoptotic pathway [Mayer et al., 2003]. These events were studied by confocal microscopy in A549 cells transfected for 48 hours with NP expressing or control plasmid. It was observed that Bax had nuclear localization in control treated cells, but in case of IAV NP-transfected cells, a significant amount of Bax moved to the mitochondrial-cytoplasmic region when examined by confocal microscopy (Fig.6A, B). Also Cyt c had peculiar perinuclear-mitochondrial localization in control treated cells, but in case of IAV NP-transfected cells it acquired a diffused cytoplasmic localization pattern indicating its release from mitochondria (Fig.7A, B).

To get a quantitative estimation Cyt c release from mitochondria and Caspase 3 activation was assessed by flow cytometry. For this A549 cells were transfected with NP expressing or control plasmids, harvested 48 hours post-transfection, stained with anti-Cyt c or anti-cleaved caspase 3 antibodies and subjected to FACS analysis. It was observed that in IAV NP expressing cells showed ≈ 3 fold more Cyt c release from mitochondria (Fig. 8) and Caspase 3 activation (Fig. 9) as compared to control cells.
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Fig.6. Ectopic expression of IAV NP leads to Bax localization to mitochondria

A549 cells were transfected with pcDNA3.1-NP or control pcDNA3.1 plasmid for 48 hours, cells were fixed and processed for immunostaining. NP was stained using anti-Myc tag primary antibody and Alexa350 conjugated secondary antibody (Blue). Bax was stained using Bax specific primary antibody and Alexa 488 conjugated secondary antibody (Green). Mitochondria were stained with CMXRos Red mitotracker dye. Panels are labeled for their respective staining. A. Shows pcDNA3.1-NP transfected cells, whereas B. Shows control pcDNA transfected cells.
Fig. 7. Ectopic expression of IAV NP leads to Cytochrome c release from mitochondria

A549 cells were transfected with pcDNA3.1-NP or control pcDNA plasmid 48 hours, cells were fixed and processed for immunostaining. NP was stained using anti-Myc tag primary antibody and Alexa488 conjugated secondary antibody (Green). Cytochrome c was stained using Cyt c specific primary antibody and Alexa 594 conjugated secondary antibody (Red). Nuclei were stained with Hoechst stain. Panels are labeled for their respective staining. A. Shows pcDNA 3.1-NP transfected cells. B. shows control pcDNA transfected cells.
A. A549 cells were transfected with pEGFP-NP or control pEGFP plasmid, 48 hours post-transfection cells were stained using Flowcellect Cytochrome c kit (Millipore). It differentially stains Cytochrome c present in mitochondria and release of Cyt c from mitochondria causes leftward shift in the histogram. GFP +ve cells were gated and among that population Cyt c +ve/-ve population were analyzed. B. Populations –ve or +ve for Cyt c staining are marked and their percentages are written in the histograms. The shown histograms are representatives of at least three independent experiments. All graphs represent mean ± standard deviation of one representative experiment (n = 3). Statistical significance was determined using Student's t test. *, p<0.05; **, p<0.01; ***, p<0.001.

Fig.8. Ectopic expression of IAV NP leads to Cytochrome c release from mitochondria
**Fig. 9. Ectopic expression of IAV NP leads to Caspase 3 activation in lung epithelial cells**

**A.** A549 cells were transfected with pEGFP-NP plasmid or control pEGFP plasmid. 48 hours post-transfection cells were harvested and stained with PE Active Caspase 3 staining kit (BD biosciences) which differentially stains cleaved form of Caspase 3, and subjected to flow cytometry. GFP +ve cells were gated out and among that population Caspase 3 +ve/-ve population were analyzed. The contour plots are representatives of at least three independent experiments.  

**B.** The select populations in the contour plots are gated and their percentage is mentioned in each plot. All graphs represent means ± standard deviations of one representative experiment (n = 3). Statistical significance was determined using Student's t test. *, p<0.05; **, p<0.01; ***, p<0.001.
4.2.2.4. NP expression in IAV-infected cells coincides with increase in apoptosis and siRNA-mediated inhibition of NP expression leads to reduction in IAV-induced cell death

To validate the role of NP in IAV-induced apoptosis, a time course study was performed in A549 cells infected with A/California/2009 (H1N1) IAV at 0.2 MOI. Increase in apoptotic events at different time points was measured by Annexin V staining and the expression patterns of viral factors known to be involved in apoptosis was checked by western blotting. IAV-induced apoptosis could be detected as early as 4 hours which increases until 24 hours, in the tested experimental conditions (Fig. 10B). While the expression of M2 and NA protein increased in the window of 12 to 24 hours (Fig. 10A, panel 3, 4), the expression of NP and NS1 started to increase at the 8 hour time point and reached their maximum at approximately 24 hours (Fig. 10A, panel 1, 2). Thus, between 8 to 12 hours’ post-infection, increase in expression of NP coincided with the increase in apoptosis. It indicates a possible role of NP in IAV induced cell death. The expression of NS1 also increased during this period; however NS1 is reported to play an anti-apoptotic role during IAV infection [Zhirnov et al., 2002]. However, the expression of Clu did not change in A549 cells during the course of IAV infection (Fig. 10A, panel 5).

To further confirm the role of NP in IAV-induced cell death, we inhibited the expression of NP using a pool of siRNAs targeting NP gene of A/California/2009(H1N1) IAV isolate in A549 cells. Anti-NP siRNA significantly inhibited expression of IAV NP; however it had a slight negative effect on the expression of other viral proteins M2 and NA (Fig.11A). This may be due to essential requirements of IAV NP for successful replication of IAV. We also tested the effect of NP inhibition on A/California/2009(H1N1) IAV-induced cells death by Annexin V staining and found significant decrease in apoptosis in case of anti-NP siRNA treated cells as compared to the control samples (Fig.11B).
Fig. 10. NP expression kinetics coincide with apoptosis induction in IAV infected cells

A. A549 cells were infected with A/California/09(H1N1) IAV at 0.2 MOI. Cells were harvested at different time intervals and cell lysates were subjected to western blotting using antibodies against NP, NS1, NA, M2, Clusterin and β-Actin proteins which are shown in panels 1, 2, 3, 4, 5 and 6 respectively. B. From above mentioned time points cells were stained with Annexin V PE stain and subjected to flow cytometry. Annexin V +ve cells are plotted on the graph. Graph represents means ± standard deviations of one representative experiment (n=3). Data shown are representatives of at least three independent experiments.
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Fig. 11. Inhibition of NP expression reduces apoptosis in IAV infected cells

A. A549 cells were transfected with siRNA pool against NP gene of Cal/09/H1N1 IAV or control non-targeting siRNA. 6 hours post-transfection, cells were infected with Cal/09/H1N1 IAV at 0.2 MOI. For western analysis, cells were harvested 24 hours post-transfection and cell lysates subjected to SDS-PAGE followed by western blotting using IAV NP, NA, M2 and β Actin specific antibodies. Panel 1 shows NP levels and Panel 2 shows loading control. Lane 1, 2 and 3 show infected cells untreated with any siRNA, treated with control siRNA and treated with anti-IAV NP siRNA respectively. B. For apoptosis study, cells were harvested 12 hours post-infection and stained with Annexin V PE and subjected to flow cytometry. Graph represents means ± standard deviations of one representative experiment (n = 3). Statistical significance was determined using Student's t test. *, p<0.05; **, p<0.01; ***, p<0.001. Data shown are representatives of at least three independent experiments.
4.2.2.5. IAV NP-induced cell death is mediated by its interaction with Clusterin

Since IAV NP interacts with Clu and induces apoptosis via the mitochondrial cell death pathway, and Clu was also involved in the same pathway, we investigated whether Clu was involved in IAV NP-induced cell death. To address this, we overexpressed Clu in A549 cells ectopically and subsequently transfected IAV NP expressing plasmid into cells. The plasmid used in the study expressed Myc tagged Clu protein and its expression was checked by western blotting (Fig.12A). We stained cells 24 hours post-NP transfection with Annexin V PE and subjected them to flow cytometric analysis. We observed that overexpression of Clu reduced IAV NP-induced cell death (Fig.13A). Moreover, when Clusterin expression was inhibited by using siRNA, IAV NP-induced cell death was enhanced as compared to the scrambled siRNA treated cells (Fig.13B). The efficacy of siRNA pool used against Clu was checked by western blotting, and the result clearly showed significant inhibition of Clu expression as compared to the control (Fig.12B). The anti-apoptotic role of Clu was also validated in A549 cells infected with IAV. For this, A549 cells were transfected with Clu expressing plasmid or treated with siRNA pool against Clusterin, followed by infection with A/California/2009(H1N1) IAV isolate. 12 hours post-infection, cells were stained with Annexin V and subjected to flow cytometry. While overexpression of Clu reduced cell death (Fig.14A), inhibition of Clu expression increased IAV-induced cells death significantly (Fig. 14B).
Fig. 12. Validation of Clu expression plasmid and anti-Clu siRNA

A. A549 cells were transfected with pCMV-Myc-Clu or control pCMV plasmid. 24 hours post-transfection cells were harvested and cell lysates were subjected to SDS-PAGE followed by western blotting using anti-Myc tag and anti-β Actin antibodies. Panel 1 shows Myc tagged Clu expression and Panel 2 shows loading control. Lane 1, 2 and 3 show cells transfected with no plasmid, pCMV control plasmid and pCMV-Myc-Clu plasmid respectively. B. A549 cells were transfected with siRNA pool against human Clu gene or control non targeting siRNA. Cells were harvested 24 hours post-transfection and cell lysates subjected to SDS-PAGE followed by western blotting using Clu and β Actin specific antibodies. Panel 1 shows Clu levels and Panel 2 shows loading control. Lane 1, 2 and 3 show cells transfected with no siRNA, control non targeting siRNA anti IAV Clu siRNA respectively.
Fig.13. Effect of Clu overexpression on apoptosis induced by NP expression and IAV infection

A. A549 cells were transfected with Clusterin expressing plasmid pCMV-Clu or control plasmid pCMV. 24 hours post-transfection, cells were transfected with pEGFP-NP plasmid or pEGFP control plasmid. Cells were harvested at 24 hours post-infection, stained with Annexin V PE stain and subjected to flow cytometry. Percentage of [GFP+Annexin V] +ve population is plotted on the graph. B. A549 cells were transfected with Clusterin expressing plasmid pCMV-Clu or control plasmid pCMV. 24 hours post-transfection, cells were infected with A/California/2009(H1N1) IAV at 0.2 MOI. Cells were harvested at 12 hours post-infection, stained with Annexin V PE stain and subjected to flow cytometry. Percentage of Annexin V +ve population is plotted on the graph. Statistical significance was determined using Student's t test. *, p<0.05; **, p<0.01; ***, p<0.001. Data shown are representatives of at least three independent experiments.
Fig. 14. Effect of siRNA mediated inhibition of Clu expression on apoptosis induced by ectopic IAV NP expression and IAV infection

A. A549 cells were transfected with siRNA pool against Clusterin gene or non-targeting control siRNA. 24 hours post-transfection, cells were transfected with pEGFP-NP plasmid. 24 hours post-infection cells were harvested and stained with Annexin V PE and subjected to flow cytometry. Percentage of [GFP+Annexin V] +ve population is plotted on the graph. B. A549 cells were transfected with siRNA pool against Clusterin gene or non-targeting control siRNA. 24 hours post-transfection; cells were infected with Cal/09/H1N1 IAV at 0.2 MOI. 12 hours post-infection cells were harvested and stained with Annexin V PE and subjected to flow cytometry. Percentage of Annexin V+ve population is plotted on the graph. All graphs represents means ± standard deviations of one representative experiment (n = 3). Statistical significance was determined using Student's t test. *, p<0.05; **, p<0.01; ***, p<0.001. Data shown are representatives of at least three independent experiments.
4.2.3. Discussion

A unique feature of influenza A viruses is the induction of massive cell death in infected host cells. Theoretically this attribute seems counterproductive for the virus; however IAVs have evolved in a manner that the induction of cell death is an essential requirement of successful and efficient virus replication [Ludwig et al., 2006]. Inhibition of pro-apoptotic molecules such as Bax or Caspases or overexpression of anti-apoptotic molecules such as Bcl2 is known to impede influenza virus replication [Olsen et al., 1996; Wurzer et al., 2003; McLean et al., 2009]. It is proposed that an accelerated rate of influenza virus replication compensates for the loss of host cells due to apoptosis. Also, influenza viruses may manipulate the apoptotic process to enhance their dissemination and kill the host cells involved in anti-viral immune response [Galluzzi et al., 2008]. Exchange of gene segments between high and low pathogenicity IAVs is known to alter their ability to induce cell death [Morris et al., 2005].

IAV nucleoprotein is a 498 amino-acid protein which binds to the viral RNA genome in a sequence independent manner [Ye et al., 2006]. Apart from protecting the viral genome it also interacts with components of the viral polymerase complex and NS1 protein [Biswas et al., 1998; Robb et al., 2011]. Its interaction with viral proteins is known to regulate transcription and replication of the viral genome [Portela et al., 2002]. IAV NP is also known to interact with various host factors and play additional roles in the virus life-cycle [Portela et al., 2002]. It has been reported that influenza virus infection induces Caspase 3 activity, which in turn cleaves the NP protein from its N-terminus [Zhimov et al., 1999], however direct role of NP in induction of Caspase 3 has not been reported yet. Activation of Caspase 3 was reported to help in the release of influenza RNP complex from the nucleus [Ludwig et al., 2006]. Apoptosis induction in IAV-infected cells is closely linked to the induction of inflammatory response. Not surprisingly, IAV proteins such as NS1 and PB1F2 which are known to cause cytokine dysregulation, are also involved in IAV-induced apoptosis [Koch et al., 2007; Varga et al., 2011]. Earlier we had shown that IAV NP modulates PKR-mediated host innate immune response [Sharma et al., 2011], and current study indicates its role in cell death.
as well. Taken together it suggests that NP could be an important virulence factor and contributor in IAV pathogenesis.

Our observation that IAV NP induces apoptosis in a dose dependent manner suggests that during a later stage of the viral life-cycle when there is a buildup of the NP protein inside the host cell, the apoptotic effect of NP will be more prominent. It has been reported that cellular localization of NP in IAV-infected cells changes from exclusively nuclear to perinuclear-cytoplasmic in early and late stages of viral replication cycle respectively. We observed that NP and Clusterin co-localize in perinuclear region of IAV-infected cells, during later stages of the infection. This supports the assumption that apoptosis induction by NP would be more prominent during the later stages of infection and is most likely mediated by NP-Clusterin interaction. PB1F2 protein of influenza viruses is known to induce cell death through mitochondrial pathway, however involvement of Cla in that process is not known yet. A/California/2009(H1N1) virus is known to possess 3 stop codons in PB1F2 ORF which leads to the translation of a truncated nonfunctional form of PB1F2 protein. Selection of the Swine flu pandemic isolate for our virus infection experiments ruled out a role for PB1F2 mediated induction of mitochondrial apoptosis.

In the current study IAV NP was found to interact with the anti-apoptotic host protein Clusterin. Cla protein has intracellular as well as secreted forms which are known to be targeted by viral proteins. Secretory form of Cla is exploited by the NS1 protein of Dengue viruses to interfere with the complement pathway [Kurosu et al., 2007]. Intracellular form of Cla is known to be upregulated in case of Hepatitis delta virus infection, as a strategy to promote cell survival [Liao et al., 2009]. We observed for the first time that Cla plays a cytoprotective role during IAV infection and is targeted by viral protein to induce apoptosis. Ectopic overexpression of Cla could reduce NP or IAV-induced apoptosis, however it did not abrogate it completely. Plausible reason for this could be activity of other apoptotic mechanisms, such as TNFR, Fas-TRAIL pathway or TGFβ activation pathway which may bypass Cla for cell death induction. Clusterin is also known to play an important role in the regulation of PI3K signaling, NFκB pathway.
and cell-cycle [Santilli et al., 2003; Ammar et al., 2008]. Implications of IAV NP interaction with Clu on these cellular processes remains to be studied.

After collective analysis of our data, we proposed a model for regulation of apoptosis in IAV infected cells by NP-Clusterin interaction (Fig.15.). According to this model NP interaction with Clusterin may prevent its interaction with Bax or dissociate the Bax-Clu complex. This will lead to Bax movement into the mitochondria, release of Cytochrome c, activation of Caspase 3 and eventually cell death. Detailed molecular mechanism of how NP interferes with Bax-Clu complex formation remains to be investigated.
Influenza A virus nucleoprotein interacts with human Clusterin protein inside the cells. Through this interaction Clu binding to Bax is prevented or Clu-Bax complex is dissociated. This leads to Bax movement into mitochondria, subsequent release of Cyt c from mitochondria, activation of Caspase 3 and eventually cell death.