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Modeling the Role of Cyclin C in Connecting Stress-Induced Mitochondrial Fission to Apoptosis

Steven J. Doyle
Rowan University

Randy Strich
Rowan University

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Rowan University
Graduate School of Biomedical Sciences

Steven J. Doyle, Randy Strich

Department of Molecular Biology, Rowan University Graduate School of Biomedical Sciences, Stratford NJ

Abstract

For normal cell function, exogenous signals must be correctly interpreted, and the proper response executed. The mitochondria are key regulatory nodes of cellular fate. For example, mitochondria undergo fission and fusion cycles depending on the energetic needs of the cell. Additionally, regulated cell death pathways also function at the mitochondria. Cyclin C is a transcriptional regulator of stress response and growth control genes. Following stress, a portion of cyclin C translocates to the cytoplasm, where it interacts with both the mitochondrial fission and apoptotic machinery. Based on these findings, we hypothesize that **cyclin C represents a key mediator linking transcription to mitochondrial fission and intrinsic regulated cell death (iRCD)**. Cyclin C has two conserved cyclin box domains each composed of five alpha-helices, termed CB1 and CB2, which mediate protein-protein interactions with regards to transcriptional regulation (Cdk8) and mitochondrial fission (Drp1), respectively. Pull down studies show that both pro-apoptotic protein Bax and fission machinery protein Drp1 interact directly with cyclin C. Cyclin C interaction is required for Bax activation and efficient iRCD; however, Drp1 is required for this interaction to occur, suggesting a role for the interaction of all three proteins. Docking simulations show cyclin C and Bax interact directly through multiple sites within amino acids 160-170 of cyclin C. Inspection of this region shows a homologous BH2 sequence, similar to that of Bcl-2 protein family members. Prior work has demonstrated that while this sequence is required for Bax binding, it is not required for binding Drp1. To further support this, preliminary modeling data suggests Drp1 interaction is mediated through the latter half of CB2, which is downstream of this sequence. Taken together, these results suggest a model that cyclin C possesses three distinct interaction domains, leading cyclin C to physically bridge the fission and apoptotic machinery and allowing the cell to properly coordinate mitochondrial dynamics with iRCD pathways.

Tripartite Functionality of Cyclin C

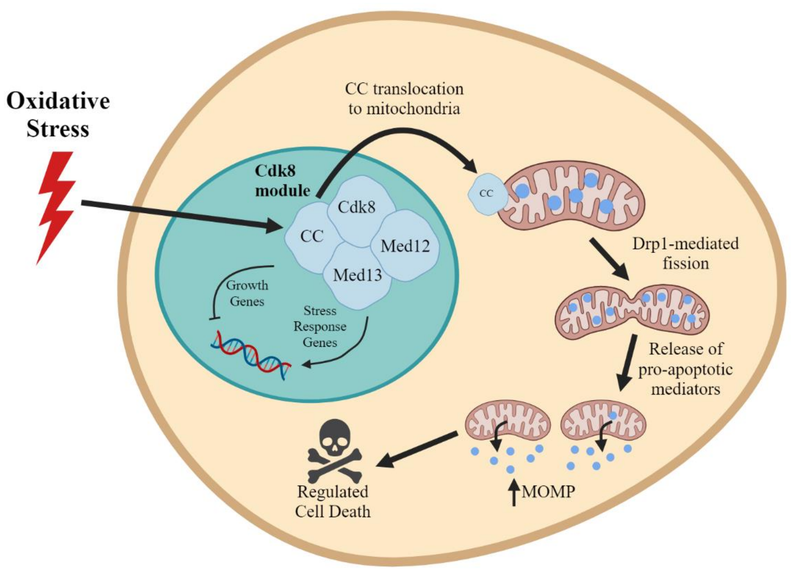


Figure 1. Cyclin C is part of the Cdk8 module or RNA polymerase II Mediator complex. Under normal growth conditions in mammalian cells, this module represses cell growth genes and promotes stress response genes. Following exposure to oxidative stress, cyclin C is translocated from the nucleus to the cytoplasm, which modifies the transcriptome. Cyclin C associates with the outer mitochondrial membrane where it is required for stress-induced mitochondrial fission and is needed to complete the intrinsic-mediated regulated cell death pathway (represented by MOMP – Mitochondrial Outer Membrane Permeability and cytochrome C release – blue circles).

Cyclin C is composed of two alpha helical CB domains

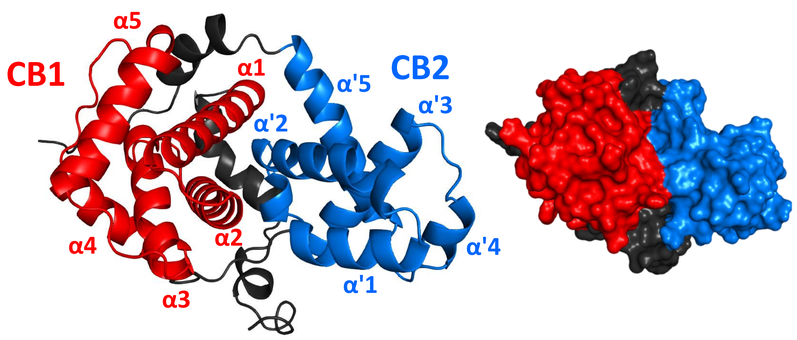


Figure 2. Structure of cyclin C. Cyclin C is composed of two five-alpha helical domains, CB1 and CB2 (red and blue, respectively). The left image represents a cartoon model with individual alpha helices numbered. The right image is a surface mesh representation of the cartoon model, showing two distinct CB domains that have distinct faces/interaction interfaces.

Cyclin C directly interacts with Bax

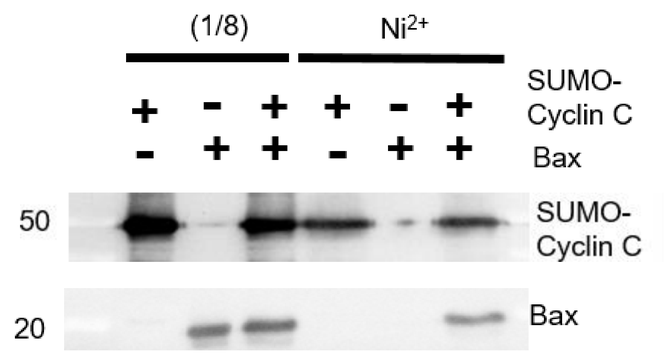
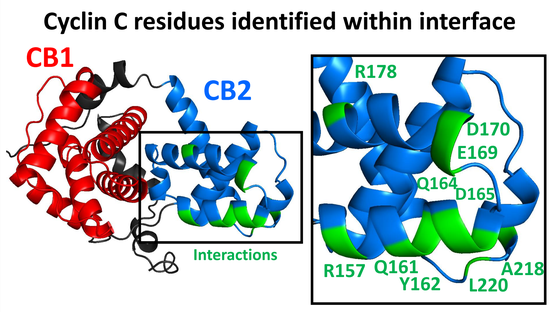


Figure 3. Cyclin C and Bax directly interact. Recombinant Bax and SUMO-cyclin C were incubated together or separately at room temperature and then passed over Ni^{2+} resin. Western blot analysis was used to probe for Bax and cyclin C. Input controls for each sample (1/8) are included. Molecular weight markers (kDa) are indicated on the left.

CC-Bax docking simulations implicate CB2 domain

Human CC-Bax		
CC		Bax
Residue	Domain	Residue
Q161	BH2	E75
Q161	BH2	T172
D165	BH2	R78
D165	BH2	Y164
Q161	BH2	S163
Q161	BH2	T169
E169	BH2	N106
A218	α'4	N106
L220	α'4/α'5	N106
Y162	BH2	G168
D165	BH2	E75
D165	BH2	T169
R178	α'2	A96
R157	α'1	L162
D170	BH2	Q28
R178	α'2	D142



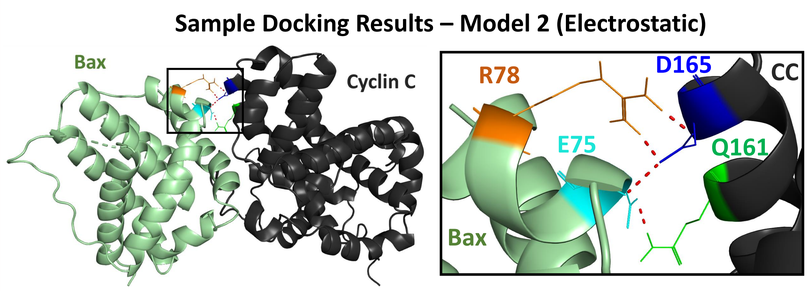


Figure 4. Cyclin C-Bax docking simulation results from ClusPro 2.0 online server analysis. Residues of interest are color coordinated in decreasing number of simulation hits: red (most), orange, yellow, light green (least). Four sets of simulation models are generated per structure pair: electrostatic favored, hydrophobic favored, balanced, and Van der Waals favored. The bottom image is a sample electrostatic favored model, highlighting example residues of interest. Red dashes represent residue interactions.

Cyclin C contains a suspected BH2 domain

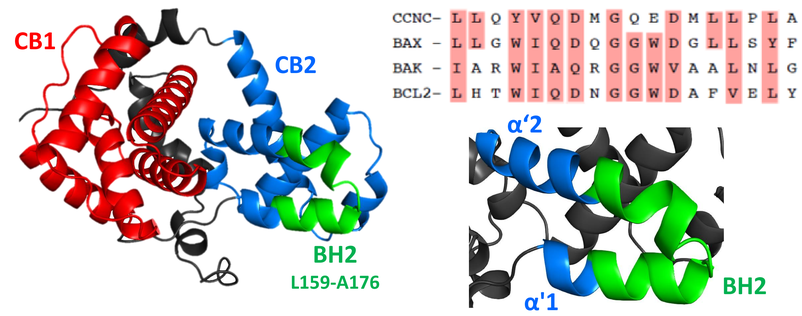


Figure 5. Cyclin C BH2 homology. The left image shows the location of the cyclin C BH2 homologous sequence (red is CB1; blue is CB2; green is BH2), located in between helices α'1 and α'2 of CB2 (zoomed in view shown in bottom right image) at residues L159-A176. The top right image shows sequence homology between cyclin C and the BH2 domains of Bcl-2 protein family members Bax, Bak, and Bcl2.

Cyclin C directly binds Drp1 through CB2 domain

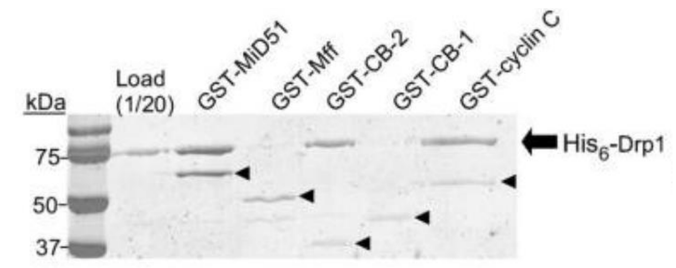


Figure 6. Cyclin C directly binds Drp1 through the CB2 domain. Binding reactions containing Drp1 incubated with either GST-MiD51 (DN118), GST-Mff(DTM), GST-cyclin box 2 (CB2), GST-cyclin box 1 (CB1), or GST-cyclin C as indicated were collected by glutathione beads and separated by SDS-PAGE followed by Coomassie staining. Drp1 and the GST fusion proteins are indicated by the arrow and arrowheads, respectively. Molecular weight markers (kDa) are indicated on the left.

Drp1 is required for Cyclin C-Bax interaction

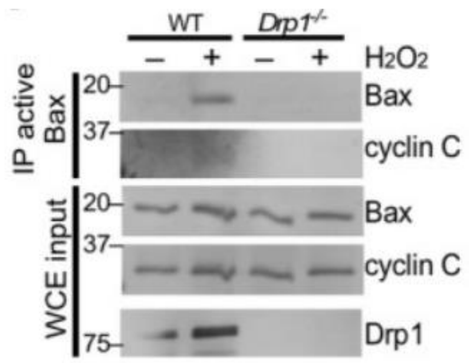


Figure 7. Drp1 is required for Cyclin C-Bax interaction. Wild-type and *Drp1*$^{-}$ MEF cultures were treated with 0.4 mM H_2O_2 as indicated and extracts prepared and immunoprecipitated with antibody recognizing the active conformation of Bax. Immunoprecipitates were subjected to Western blot analysis and probed for total Bax or cyclin C. Whole cell extracts (WCE) were subjected to Western blot analysis as indicated to control for protein concentrations in extracts. Molecular weight markers (kDa) are indicated on the left.

Conclusions

Cyclin C directly binds both Bax & Drp1, but Drp1 is required for cyclin C-Bax interaction

Simulations show residues L160-D170 of cyclin C are responsible for CC-Bax interaction

Cyclin C contains a region with high homology to Bcl-2 protein family BH2 domains, which matches simulation results and may serve as the binding interface for cyclin C-Bax

Results support a model that cyclin C physically bridges fission/apoptotic machinery allowing the cell to properly coordinate mitochondrial dynamics with PCD pathways