Published in final edited form as:

Angew Chem Int Ed Engl. 2015 October 5; 54(41): 12064–12068. doi:10.1002/anie.201506346.

Optogenetic Apoptosis: Light-Triggered Cell Death

Robert M. Hughes, **David J. Freeman**, **Kelsey N. Lamb**, **Rebecca M. Pollet**, **Weston J. Smith**, and **David S. Lawrence**

Department of Chemistry, Division of Chemical Biology and Medicinal Chemistry, and Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599 (USA)

Robert M. Hughes: rhughes@email.unc.edu; David S. Lawrence: lawrencd@email.unc.edu

Abstract

An optogenetic Bax has been designed that facilitates light-induced apoptosis. We demonstrate that mitochondrial recruitment of a genetically encoded light-responsive Bax results in the release of mitochondrial proteins, downstream caspase 3 cleavage, changes in cellular morphology, and ultimately cell death. Mutagenesis of a key phosphorylatable residue or modification of the C-terminus mitigates background levels (dark) of apoptosis due to Bax overexpression. The mechanism of optogenetic Bax-mediated apoptosis was explored using a series of small molecules known to interfere with various steps in programmed cell death. Optogenetic Bax appears to form a mitochondrial apoptosis-induced channel analogous to that of endogenous Bax.

Keywords

apoptosis; optogenetics; protein engineering; synthetic biology; photochemistry

Light has been used to control the biochemistry of cells, manipulate the behavior of organisms, and treat diseases such as cancer.[1] Recently, the application of genetically-encoded light responsive proteins for controlling the biochemistry, and therefore the behavior, of cells and organisms has elicited widespread attention.[2] Indeed, a database search revealed that the term “optogenetics” is found in well over 800 funded NIH grants (NIH RePorter) and in more than 200 reviews (PubMed). However, the majority of studies to date have employed light-responsive proteins appropriated from microorganisms (e.g. ion channels) that have been applied to the arena of neuroscience.[3] By contrast, relatively few light-activatable analogs of endogenous mammalian proteins have been successfully engineered.[4] Indeed, strategies for the design of optogenetic proteins have been described as “still in the development stage”.[5] With this challenge in mind, we report the design, construction, and application of a genetically encoded inducer of programmed cell death (apoptosis).

Apoptosis is a highly regulated self-contained response to stress-inducing environmental challenges. This dynamic process is modulated by a diverse array of proteins that, upon malfunction, contribute to diseases that range from cancer to neurological disorders.

Correspondence to: Robert M. Hughes, rhughes@email.unc.edu; David S. Lawrence, lawrencd@email.unc.edu.

Optogenetic analogs of apoptotic protein participants could potentially be used to exogenously modulate (and thereby interrogate) life/death decisions with exquisite biochemical, spatial, and temporal precision. For example pro- and anti-apoptotic members of the Bcl-2 family of proteins work in concert to control outer mitochondrial membrane (OMM) permeabilization, a critical event during apoptosis.[6] In addition, a large number of non-Bcl-2 proteins are recruited to the OMM, and assist the Bcl-2 proteins in the decision making process that governs cell fate.[7] We describe herein the design and behavior of an optogenetic Bax. Bax, a key effector of apoptosis, translocates from the cytosol to the OMM in response to apoptotic-inducing insults.[8] Once OMM associated, Bax oligomerizes and subsequently participates in the formation of the mitochondrial apoptosis-induced channel (MAC) through which cytochrome c is released into the cytosol. Key elements of our optogenetic Bax design strategy include (a) the application of light to induce a Bax concentration jump at the OMM surface to promote Bax oligomerization and (b) the introduction of mutations to reduce background (i.e. dark) activity of Bax. “Dark activity” is a common problem that has bedeviled the acquisition of well-behaved optogenetic species.[5] We demonstrate herein the light-triggered recruitment of several Bax constructs to mitochondria, the permeabilization of the OMM via release of Smac1 and subsequent cleavage of caspase 3, and that the mechanism of optogenetic Bax-driven apoptosis appears to recapitulate that of the endogenous protein.

We built upon previous work from our lab, which employed a photo-heterodimerizer system,[9] to engineer Bax constructs that are recruited to mitochondria upon illumination (Scheme 1). In brief, Cry2 (derived from a flavin-containing cryptochrome photoreceptor) undergoes a rapid conformational change upon brief exposure to 488 nm.[10] In the light-activated state, Cry2 binds to a protein partner (Cib) to form a transient Cry2-Cib complex.[10] For our initial studies, we prepared a Tom20-Cib-GFP construct, where Tom20 is a fragment of a protein that is anchored to the surface of the OMM.[11] This places the Cib component at the intracellular site that endogenous Bax associates with in response to apoptotic-inducing environmental events.[11] We first examined the light-triggered migration of a Cry2-mCh (mCherry) construct from the cytoplasm to the OMM by simultaneously expressing it along with Tom20-Cib-GFP in multiple cell lines (Cos7, HeLa, MtLn3; Fig. 1; Supporting Fig. 1, Supporting Movies 1 & 2). A single 10 ms pulse proved to be sufficient to alter the spatial distribution of Cry2-mCh from diffuse cytoplasmic to mitochondrial within 30 s. The dark half-life of the mitochondrial localized post-illuminated Cry2-Cib construct is 140 s.

In order to address the issue of dark activity, which typically compromises the design and acquisition of optogenetic proteins, we prepared variants of Bax that preclude association with mitochondria. In particular, we focused our attention on the C-terminus of the protein as well as the S184 residue, both of which play key roles in regulating the cytosolic/mitochondrial distribution of Bax. *A free C-terminus is known to be essential for Bax mitochondrial migration in response to an apoptotic stimulus.[12] In addition, the phosphorylation status of S184 is known to control Bax localization, where phospho184 Bax is cytoplasmic and the dephospho-form is mitochondrial.[13]* With these features in mind, we prepared the following: (1) Free C-terminus Bax constructs Cry2-mCh-Bax^{WT}, the S184E phospho-mimetic Cry2-mCh-Bax^{S184E}, and the nonphosphorylatable S184V Cry2-mCh-

Bax^{S184V}; (2) Substituted C-terminus constructs Bax^{WT}-Cry2-mCh, Bax^{S184E}-Cry2-mCh, and Bax^{S184V}-Cry2-mCh (Table 1, Supporting Fig. 2). The phospho-mimetic mutant Ser184Glu is reported to generate a cytosolic Bax, whereas introduction of a hydrophobic residue (Ser184Val) induces constitutive mitochondrial localization of Bax.[13] The approximate predicted masses of all the constructs were confirmed via western blot analysis (Supporting Fig. 2).

We tested the free C-terminus constructs Cry2-mCh-Bax^{WT}, Cry2-mCh-Bax^{S184E}, Cry2-mCh-Bax^{S184V} in the dark. All behaved as predicted where the S184E is cytoplasmic and the S184V is mitochondrial, with numerous peri-mitochondrial speckles. The latter is consistent with the formation of pre-apoptotic, mitochondrially associated Bax clusters[1] (Supporting Fig. 3). The Cry2-mCh-Bax^{WT} is both mitochondrial and cytoplasmic, consistent with the fact that WT Bax is known to exist in equilibrium between mitochondria and cytosol.[14] We also examined the C-substituted Bax constructs (Bax-Cry2-mCh) in the dark and found that all three are cytoplasmic. This demonstrates the profound effect of Bax C-substitution, which even overwhelms the S184V mutation that ordinarily would drive Bax to the mitochondria.[13] We subsequently analyzed the response of these Bax constructs to treatment with staurosporine (STS), a broad spectrum protein kinase inhibitor that induces apoptosis. Within 2 h, constructs with Bax at the C-terminus (Cry2-mCh-Bax) are recruited to the mitochondria, whereas the corresponding Bax-Cry2-mCh proteins remain cytoplasmic (Supporting Fig. 4). The inability of the Bax-Cry2-mCh constructs to undergo STS-induced recruitment to the mitochondria demonstrates that Bax, with an exposed free C-terminus, is absolutely essential for mitochondrial association. It further suggests that blocking the C-terminal domain may be an effective way of limiting background cell death associated with Bax overexpression.

We subsequently examined the light initiated recruitment of both Cry2-mCh-Bax^{S184E} and Bax^{S184E}-Cry2-mCh constructs to mitochondria in HeLa cells. We observed rapid cell collapse upon mitochondrial recruitment of Cry2-mCh-Bax^{S184E} over a 1 h time course of illumination (10 ms every 2 min) and imaging (Fig. 2). In an analogous fashion, cells harboring Bax^{S184E}-Cry2-mCh collapse and die upon illumination. However, this occurs over a significantly longer time period (2 – 3 h) than that required for Cry2-mCh-Bax^{S184E} (Supporting Movie 2). We also performed the following control experiments: (1) Mitochondrial recruitment of the construct lacking Bax, namely Cry2-mCh, under identical conditions does not result in a loss in cell viability (Supporting Movie 2/Fig. 2). In addition (2), we tested the effect of recruiting Cry2-mCh-Bax^{S184E} to the plasma membrane using Cib-GFP-CAAX, where CAAX serves as a plasma membrane localization motif. Sustained recruitment of Cry2-mCh-Bax^{S184E} to the plasma membrane does not result in cell collapse, indicating that the activity of optogenetic Bax is specific to the OMM (Supporting Movie 3/ Supporting Fig. 5).

We also examined the effect of light-induced mitochondrial recruitment of Bax on long term cell viability (24, 48 h) using a trypan blue exclusion assay (Supporting Figs. 6 – 7).[15] Since this particular experiment required a relatively large cell population, we employed an in-house built LED array for illumination purposes (as opposed to illumination under the microscope). The photon flux produced by our LED array (6.9 ± 0.4 μmol m^{-2} s^{-1}) is

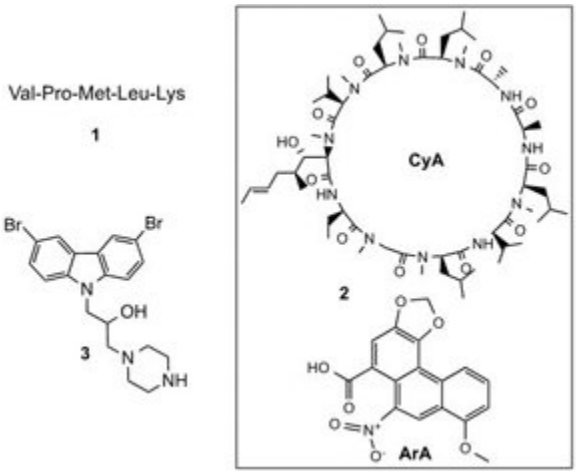
significantly less than that received by cells under the microscope (296 ± 1 μmol m^{-2} s^{-1}), and thus we employed a more aggressive illumination protocol (2 s every 2 min for 2 h). 48 h after light exposure, >60% of the Cry2-mCh-Bax^{S184E} cells had detached from the plate and nearly half of the *remaining* adherent cells incorporated trypan blue (and were therefore non-viable). By contrast, only 11% of the cells transfected with the Cry2-mCh control and illuminated were non-viable and only 8% of the cells in the non-transfected control were non-viable.

We also explored the minimum number of light pulses required to promote mitochondrial association. We found that, while a single 100 ms pulse is not sufficient to induce apoptosis, differences in behavior between the various constructs are nonetheless apparent even under these conditions (Supporting Fig. 8). Specifically, the mitochondrial residence time (half-life) of the Cry2-mCh-Bax fusions is significantly longer (200 ± 10 s) than their Bax-Cry2-mCh counterparts (130 ± 10 s) (Table 1, Supporting Fig. 8). Subsequent studies using a series of five 10 ms pulses, spaced 5 min apart, revealed that Cry2-mCh-Bax^{S184E} remains associated with the mitochondria, even 30 min after the final 10 ms pulse (Supporting Fig. 9). By contrast, the Bax^{S184E}-Cry2-mCh construct, under analogous illumination conditions, fails to be retained by the mitochondria once illumination is halted. Given the fact that cells harboring Cry2-mCh-Bax^{S184E} suffer brisk apoptosis (1 h) relative to cells containing Bax^{S184E}-Cry2-mCh (2 – 3 h), it is tempting to speculate that the Cry2-mCh-Bax^{S184E} construct undergoes more efficient mitochondrial oligomerization and/or OMM insertion than its Bax^{S184E}-Cry2-mCh counterpart.

We also assessed whether anticipated apoptotic events, downstream of Bax recruitment to the mitochondria, are triggered following illumination. Proteolytic conversion of the inactive caspase-3 zymogen to the active caspase-3 fragment (17 kDa) serves as a barometer of the cell's commitment to the execution phase of apoptosis.[16] Since the 17 kDa fragment was assayed via western blot analysis (as opposed to microscopy), we once again resorted to bench-top illumination using the in-house built LED array. Transfected Cos7 cells were illuminated for 2 s, every 2 min, over the course of 1, 2, or 3 h. As anticipated, both Bax^{WT} and Bax^{S184E}, positioned at either the N- or the C-terminus (Bax-Cry2-mCh and Cry2-mCh-Bax, respectively), mediate the light triggered formation of the 17 kDa caspase fragment (Supporting Fig. 10). By contrast, an increase in 17 kDa fragment formation was not observed with light alone or upon light-triggered migration of Cry2-mCh to the mitochondria. *We also note that the Bax^{WT} constructs induce a 50% higher caspase-3 fragment background in the dark than the corresponding Bax^{S184E} mutants. This undesired dark activity is a common problem with optogenetic proteins and highlights the importance of employing mutant constructs whose endogenous activities are biochemically compromised*. The observed dark activity for the Bax^{WT} construct may be due to the equilibrium between the cytosol and the mitochondria for the WT protein that exists even in the absence of an apoptotic insult.[14] Of the four Bax constructs, Bax^{S184E}-Cry2-mCh is the most effective, producing a 3.5-fold increase in caspase-3 fragment generation upon illumination, while maintaining a modest caspase-3 fragment background in the dark (Supporting Fig. 10).

To further assess whether the Bax constructs are acting in a manner consistent with Bax-dependent apoptosis, we examined the mitochondrial release of Smac1, using a $Smac1^{1–60}$-GFP fusion. Smac1 resides in the mitochondria intermembrane space, and is known to co-disperse with cytochrome C during Bax-induced apoptosis.[17] As expected, we observed the light-triggered loss of Smac1 from mitochondria during Bax-induced cellular collapse (Fig. 3, Supporting Movie 4). This release is not gradual, but occurs rapidly as cells transition from adherent to non-adherent, an observation consistent with the fact that specific critical apoptotic biochemical events can occur within minutes.[18]

Finally, we tested the effects of several inhibitors on cells containing the optogenetic Bax constructs. V5 (**1**) is a cell permeable peptide that inhibits the mitochondrial translocation of Bax (endogenous or overexpressed) by disrupting the interaction between Bax and Ku70.[19] At a concentration (200 μM) sufficient to block the cytosol-to-mitochondrial migration of overexpressed wild type Bax,[19] optogenetic Bax still induces rapid cell collapse (Supporting Movie 5/Supporting Table 1). V5's ineffectiveness is expected since light-induced migration of optogenetic Bax to the mitochondria occurs independently of the Ku70 mechanism employed by its' endogenous counterpart. We also tested a cocktail of cyclosporin A and aristolochic acid (5 μM CyA/50 μM ArA; **2**), which disrupts the Ca^{2+} MPT, but not the Bax/Mg^{2+} MPT.[20] Once again, as anticipated, this cocktail fails to inhibit the light-mediated pro-apoptotic action of optogenetic Bax (Supporting Movie 5/Supporting Table 1). Finally, we examined the action of an inhibitor (**3**)[21] that blocks the mitochondrial apoptosis-induced channel (MAC), which is formed by Bax and is the conduit through which cytochrome C is released. We found that not only is the MAC channel inhibitor effective at blocking light-induced apoptosis (Supporting Movie 5/Supporting Table 1), but that the inhibitory action is also titratable over a 1 – 10 μM concentration range of inhibitor **3** (Supporting Movie 6/Supporting Table 2).[21] These results are consistent with the reported near complete inhibition of mitochondrial permeability by **3** (10 μM)[21] and strongly suggests that optogenetic Bax forms a MAC analogous to that of the endogenous protein.



The therapeutic interest in Bax-mediated apoptosis encompasses a wide array of diseases, from cancer to neuropathologies. As a consequence, a variety of tools have been developed

to explore and/or perturb apoptotic pathways, including small molecule inhibitors and activators.[22] Light has been used, in conjunction with photosensitizers[23] or genetically expressed proteins[24], to induce reactive oxygen species and cell death. In addition, a variety of light-responsive small molecules have been described that trigger cell death via an array of miscellaneous mechanisms.[25] Optogenetics offers the ability to use light to initiate an action, with exquisite temporal, spatial and *biochemical* precision, which provides the ability to correlate an investigator-defined biochemical event with cellular behavior. With this in mind, there is intense interest in exploring the aberrant Bax-mediated activity associated with Alzheimer's Disease (AD) and developing appropriate animals models that can be used to assess therapeutic efficacy.[26} Furthermore, the geographical course of AD, from the hippocampus out to the cortex, is a spatially well-defined phenomenon; one that optogenetics may prove to be uniquely capable of addressing.

In summary, we've developed an optogenetic design strategy that is based on the aberrant mitochondrial migration behavior of Bax mutants in response to pro-apoptotic insults. This strategy furnishes constructs that display minimal dark activity, an otherwise common problem associated with optogenetic engineering. Finally, we've identified two constructs that exhibit distinct properties: both Cry2-mCh-Bax^{S184E} and Bax^{S184E}-Cry2-mCh elicit light-mediated cell death, however the former also migrates to the mitochondria in response to conventional pro-apoptotic signals (exposure to STS) whereas the latter remains cytoplasmic under these conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the National Institutes of Health for financial support (1R01CA159189). We thank Mr. Colin O'Banion for providing the human Tom20 sequence and Professor Mohanish Deshmukh and Mr. Matt Geden for helpful discussions.

References

1. (a) Rkein AM, Ozog DM. Dermatol. Clin. 2014; 32:415–425. [PubMed: 24891062] (b) Klan P, Solomek T, Bochet CG, Blanc A, Givens R, Rubina M, Popik V, Kostikov A, Wirz J. Chem. Rev. 2013; 113:119–191. [PubMed: 23256727] (c) Lee HM, Larson DR, Lawrence DS. ACS Chem. Biol. 2009; 4:409–427. [PubMed: 19298086] (d) Brieke C, Rohrbach F, Gottschalk A, Mayer G, Heckel A. Angew. Chem. Int. Ed. Engl. 2012; 51:8446–8476. [PubMed: 22829531]
2. Gautier A, Gauron C, Volovitch M, Bensimon D, Jullien L, Vriz S. Nature Chem. Biol. 2014; 10:533–541. [PubMed: 24937071]
3. Hausser M. Nat. Methods. 2014; 11:1012–1014. [PubMed: 25264778]
4. (a) O'Neill PR, Gautam N. Photochem. Photobiol. Sci. 2015(b) Weitzman M, Hahn KM. Curr. Opin. Cell Biol. 2014; 30:112–120. [PubMed: 25216352]
5. Yin T, Wu YI. Pflugers Archiv : Euro. J. Physiol. 2013; 465:397–408. [PubMed: 23417571]
6. Youle RJ, Strasser A. Nat. Rev. Mol. Cell. Biol. 2008; 9:47–59. [PubMed: 18097445]
7. (a) Franklin-Tong VE, Gourlay CW. Biochem. J. 2008; 413:389–404. [PubMed: 18613816] (b) Chua BT, Volbracht C, Tan KO, Li R, Yu Victor C, Li P. Nature Cell Biol. 2003; 12:1083–1089. [PubMed: 14634665]
8. Walensky LD, Gavathiotis E. Trends Biochem. Sci. 2011; 36:642–652. [PubMed: 21978892]

9. Hughes RM, Lawrence DS. Angew. Chem. Intl. Ed. Engl. 2014; 53:10904–10907.
10. Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL. Nat Methods. 2010; 7:973–975. [PubMed: 21037589]
11. (a) Bellot G, Cartron PF, Er E, Oliver L, Juin P, Armstrong LC, Bornstein P, Mihara K, Manon S, Vallette FM. Cell Death. Differ. 2007; 14:785–794. [PubMed: 17096026] (b) Endo T, Kohda D. Biochim. Biophys. Acta. 2002; 1592:3–14. [PubMed: 12191763]
12. Valentijn AJ, Upton J-P, Bates N, Gilmore AP. Cell Death. Differ. 2008; 15:1243–1254. [PubMed: 18437166]
13. Nechushtan A, Smith CL, Hsu Y-T, Youle RJ. EMBO J. 1999; 18:2330–2341. [PubMed: 10228148]
14. Schellenberg B, Wang P, Keeble JA, Rodriguez-Enriquez R, Walker S, Owens TW, Foster F, Tanianis-Hughes J, Brennan K, Streuli CH, Gilmore AP. Mol. Cell. 2013; 49:959–971. [PubMed: 23375500]
15. Perry SW, Epstein LG, Gelbard HA. Biotechniques. 1997; 22:1020–1021. [PubMed: 9187742]
16. Boatright KM, Salvesen GS. Cur. Opin. Cell Biol. 2003; 15:725–731.
17. Muñoz-Pinedo C, Guío-Carrión A, Goldstein JC, Fitzgerald P, Newmeyer DD, Green DR. Proc. Natl. Acad. Sci. USA. 2006; 103:11573–11578. [PubMed: 16864784]
18. Green DR. Cell. 2005; 121:671–674. [PubMed: 15935754]
19. Gomez J, Matsuyama S. Methods Mol. Biol. 2011; 683:465–471. [PubMed: 21053150]
20. Eskes R, Antonsson B, Osen-Sand A, Montessuit S, Richter C, Sadoul R, Mazzei G, Nichols A, Martinou JC. J. Cell Biol. 1998; 143:217–224. [PubMed: 9763433]
21. Bombrun A, Gerber P, Casi G, Terradillos O, Antonsson B, Halazy S. J. Med. Chem. 2003; 46:4365–4368. [PubMed: 14521400]
22. Roy MJ, Vom A, Czabotar PE, Lessene G. Brit. J. Pharmacol. 2014; 171:1973–1987. [PubMed: 24117105]
23. Craig RA, McCoy CP, Gorman SP, Jones DS. Exp. Opin. Drug Deliv. 2015; 12:85–101.
24. Wojtovich AP, Foster TH. Redox Biol. 2014; 2:368–376. [PubMed: 24563855]
25. For recent examples see Borowiak M, Nahaboo W, Reynders M, Nekolla K, Jalinot P, Hasserodt J, Rehberg M, Delattre M, Zahler S, Vollmar A, Trauner D, Thorn-Seshold O. Cell. 2015; 162:403–411. [PubMed: 26165941] and Shell TA, Shell JR, Rodgers ZL, Lawrence DS. Angew. Chem. 2014; 126:894–897. Angew. Chem. Int. Ed. 2014, 53, 875 – 878.
26. Obulesu M, Lakshmi MJ. Neurochem. Res. 2014; 39:2301–2312. [PubMed: 25322820]

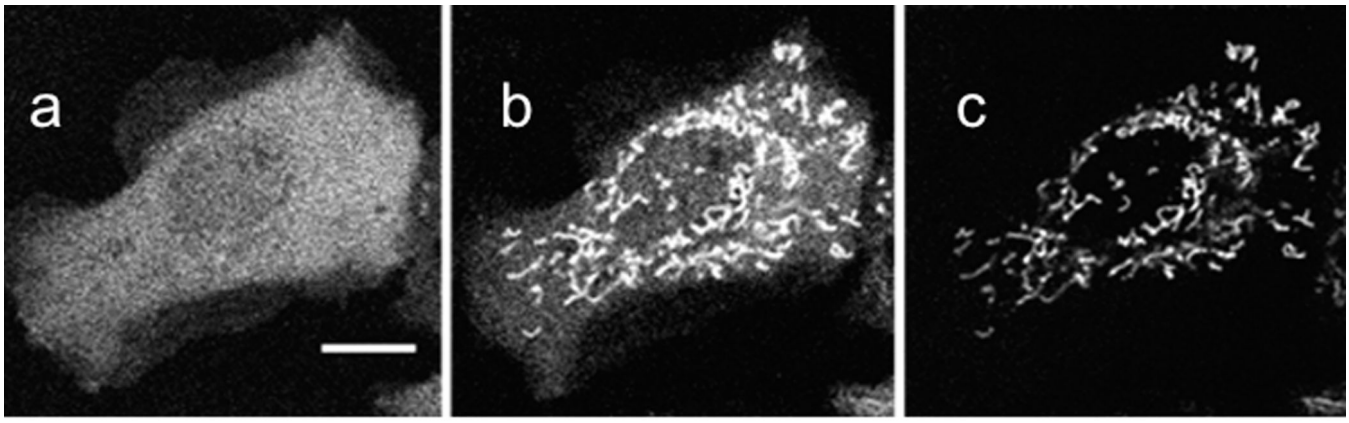


Figure 1.

Visualization of recruitment of Cry2-mCh to the mitochondria in MtLn3 cells via confocal microscopy. Cells are shown (a) prior to and (b) following 488 nm illumination. The localization construct (Tom20-Cib-GFP) is shown in (c). See Supporting Fig. 1 for results with HeLa and Cos7 cell lines.

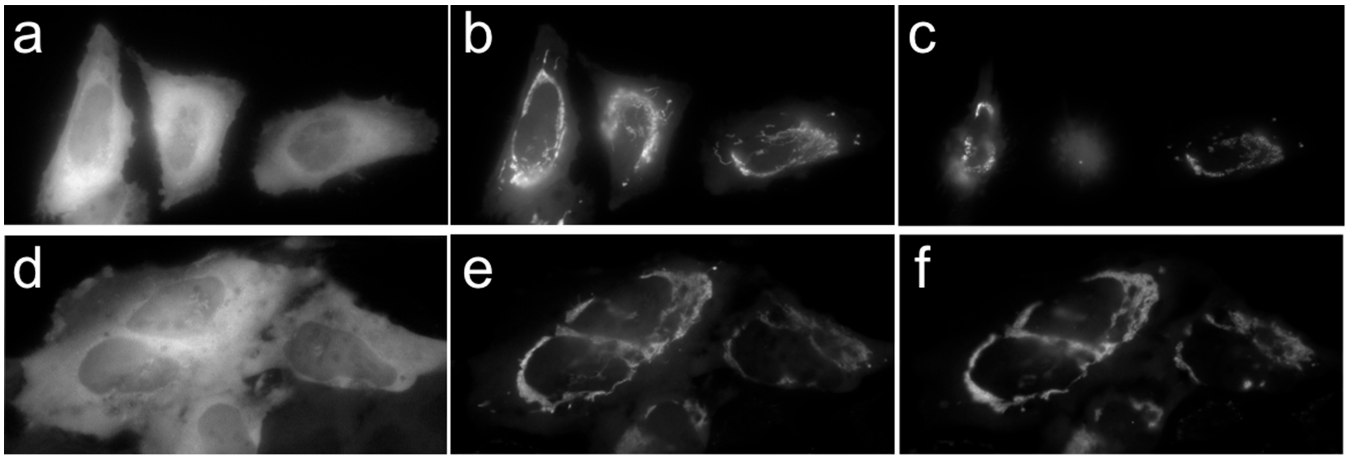


Figure 2.

Visualization of Cry2-mCh-Bax^{S184E} (a – c) and Cry2-mCh (d – f) light-triggered recruitment to mitochondria in HeLa cells and the attendant cell morphology. Cells are shown prior to illumination (a, d), 2 min (b, e), and 18 min after first light pulse (10 ms pulse every 2 min). Cry2-BaxS184E cells undergo rapid shrinkage (b) and eventual detachment (c; see middle cell), while Cry2-mCh cells maintain an adherent morphology throughout the light course.

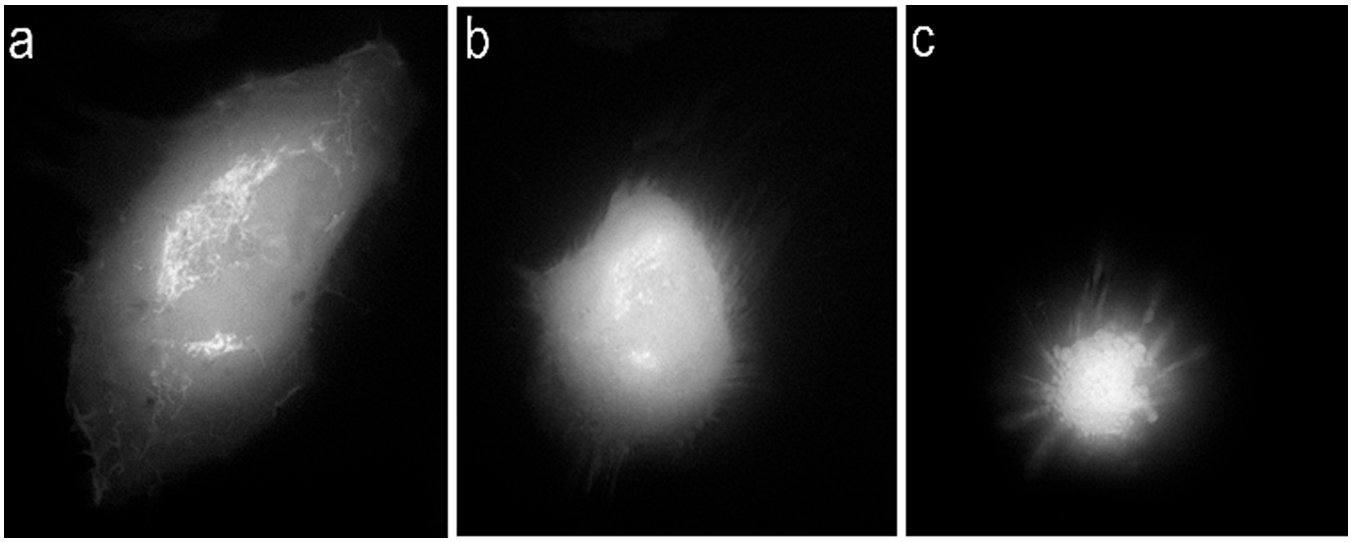
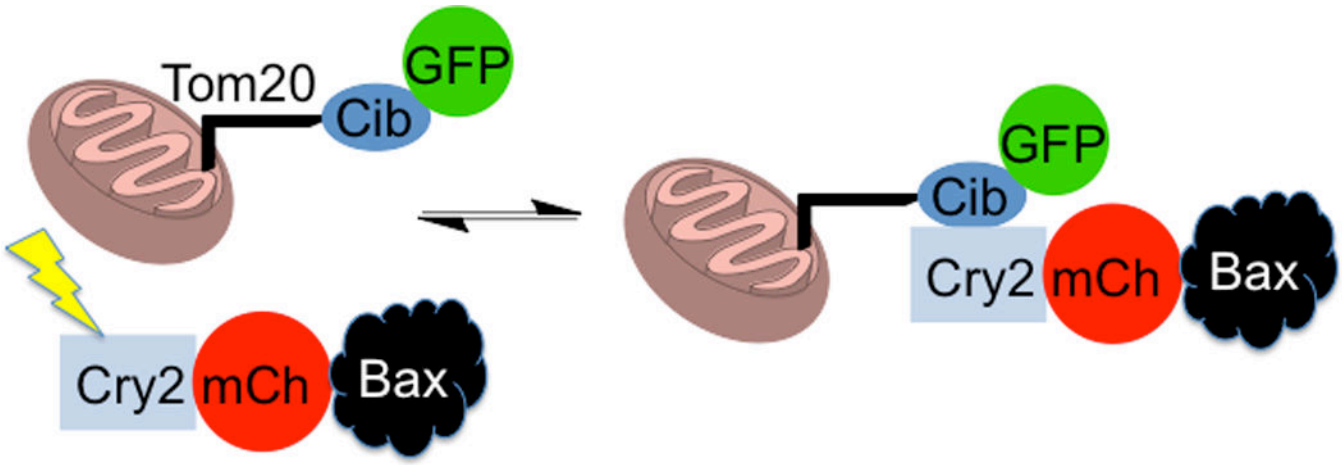


Figure 3.

Visualization of Smac1-GFP release from mitochondria of Cry2-mCh-Bax^{S184E}/Tom20-Cib/Smac1-GFP transfected HeLa cells. (a) Initial localization of Smac1 is mitochondrial. A 10 ms pulse was then applied every 2 min. At the (b) 12 and (c) 14 min time points Smac1 localization is distinctly less mitochondrial during cellular collapse.



Scheme 1.

Design of a genetically encoded optogenetic Bax. A Bax mutant (black) is appended to the light-responsive Cry2 (light blue). In the absence of light, the Bax construct remains cytoplasmic. Upon illumination at 488 nm, Cry2 associates with mitochondria-bound Cib (blue), furnishing a high effective Bax concentration, promoting Bax oligomerization, mitochondrial pore transition, Smac1 release, caspase 3 fragment formation, and eventual cell death.

Table 1

Optogenetic Bax constructs and their subcellular location.

Construct	Dark	Dark + STS[a]	Mitochondrial Half-Life[b]
Cry2-mCh-Bax^{WT}	Mitochondrial + Cytoplasmic	Mitochondrial	200 ± 10 s
Cry2-mCh-Bax^{S184E}	Cytoplasmic	Mitochondrial	210 ± 10 s
Cry2-mCh-Bax^{S184V}	Mitochondrial	Mitochondrial	N.D. [c]
Bax^{WT}-Cry2-mCh	Cytoplasmic	Cytoplasmic	130 ± 10 s
Bax^{S184E}-Cry2-mCh	Cytoplasmic	Cytoplasmic	130 ± 10 s
Bax^{S184V}-Cry2-mCh	Cytoplasmic	Cytoplasmic	N.D.

[a] STS = staurosporine.

[b] Mitochondrial residence time following a single 100 ms 488 nm light pulse,

[c] N.D. = Not determined.