



# Evidence that core histone H3 is targeted to the mitochondria in *Brassica oleracea*

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## Abstract

The core histone proteins H2A, H2B, H3 (histone H3) and H4 are known to form nucleosomes with nuclear DNA, but are historically considered to be absent from mitochondria. We suggest that H3 is a dual-targeted protein, found in mitochondria as well as N (nuclei). WoLF PSORT and MitoProt analyses of H3 sequences revealed mitochondrial targeting signals, and immunohistochemistry indicated mitochondrial distribution. Western blots of *Brassica oleracea* cv. Botrytis (cauliflower) mitochondrial extracts were positive for H3, when the primary antibody was against the conserved C-terminus. MS/MS (tandem mass spectrometry) analyses confirmed the Western blot data. Interestingly, Western blots of the same mitochondrial extracts were almost completely negative for H3 when the primary antibodies were highly specific for the N-terminal tail region of H3, suggesting that these antibodies are blocked by a modification of the tail of the H3 that occurs predominantly in the mitochondria, but not in the nucleus. Modifications of the tail of core H3 are known to help control nuclear genes. Future studies of the possible functions of mitochondrial H3 could lead to a greater understanding of the ability of a cell to synchronize nuclear and mitochondrial gene expression.

Keywords: dual-targeting; evolution; histone H3; modification; nuclear mitochondrial coordination

## 1. Introduction

H3 (histone H3) protein is a highly conserved, structurally and functionally important component of chromosomes, and it is found in the nucleus of all known eukaryotic organisms. Historically, histone proteins are considered to be absent from mitochondria (Salganik et al., 1991; Kutsyi et al., 2005); indeed, histones are defined as nuclear proteins used for stabilizing and regulating nuclear DNA. Eukaryotic nuclear DNA is known to wrap around each octamer of the core histone proteins H2A, H2B, H3 and H4, forming a structure called a nucleosome, and the linker histone, H1, binds to the DNA between nucleosomes. When cells prepare to enter mitosis or meiosis, the nucleosomes pack together tightly, forming compact chromosomes; while during transcription, histone modifications make DNA accessible, by allowing genes to be loosened from histone proteins (Bartova et al., 2008).

While the structure and function of nuclear nucleosomes have been studied extensively, much less is known about the organization and regulation of mitochondrial DNA (Binder and Brennicke, 2003). DNA–protein complexes called nucleoids have been described in yeast and in humans (Newman et al., 1996; Kaufman et al., 2000; Garrido et al., 2003), and two mitochondrial histone-like proteins have been well-studied: HM in yeast (Caron et al., 1979), and mtSSB in several animal species (Garrido et al., 2003). In *Arabidopsis thaliana*, At3g18580 and At3g51880 have been identified as putative orthologues of nucleoid proteins (Elo et al., 2003).

Here, we present Western blot data (Figure 2), tandem mass spectrometry evidence (Table 1), and WoLF PSORT (Horton et al.,

2007), MitoProt II (Claros and Vincens, 1996) prediction values (Table 2) and immunohistochemistry results (Figure 4) that suggest the presence of true H3 protein in mitochondria of cauliflower *Brassica oleracea*. This finding is discussed in relation to cellular coordination of metabolism, transcription, replication and even apoptotic signals between the mitochondria and the nucleus. We speculate that this finding may also lead to advancements in understanding the evolution of nuclear and mitochondrial genomes and proteomes.

Many different enzymatic modifications of the core histones are known to contribute to transcription regulation and chromatin structure in the nucleus (Strahl and Allis, 2000; Bartova et al., 2008). Histone modifications are often coordinated with the action of soluble transcription factors; for example, hBm (Soutoglou and Talianidis, 2002), HNF1- $\alpha$  (Parrizas et al., 2001), HNF4- $\alpha$  (Hatzis and Talianidis, 2002), RAR/RXR (Dilworth et al., 2000), IFN- $\beta$  (Agalioti et al., 2000), p53 (Nguyen et al., 2005) and CTF-1 (Alevizopoulos et al., 1995) are all transcription factors whose activities are coordinated with particular histone modifications. Thus, although the importance of combining the activation of transcription factors with the modification of H3 is well established for nuclear gene regulation, mitochondrial DNA is not known to associate with H3 or with any of the other core histones found in the nucleus.

Coordination of nuclear and mitochondrial gene function must be under cellular control; however, the mechanism of this coordination remains elusive. Several important nuclear transcription factors including human HMGA1 (Dement et al., 2005), plant Whirly proteins (Krause et al., 2005) and a member of the AP2/EREBP family in plants (Schwacke et al., 2007) have all been shown to be dual-targeted to nuclei and to mitochondria, possibly

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**Abbreviations:** DCIP, 2,6-dichlorophenol indophenol; H3, histone H3; HRP, horseradish peroxidase; LC-MS/MS, liquid chromatography tandem mass spectrometry.

**Table 1 LC-MS/MS protein identification**

Identity of mitochondrial protein confirmed as H3 by tryptic peptide sequence comparison with known sequence of H3 (accession number Q43566). Matched peptides (37% sequence coverage) shown in bold. Data from The Rockefeller University Protein/DNA Technology Center.

**ASWTGGKGXR** **KQXATKAARX** **SAPATGGVVK** **PHRFRPGTVA**  
**LREIRRYQKS** **XELLIRKXPF** **QRXVREIAQD** **FKTDLRFQXS**  
**AVAALQEAAE** **SYLVGLFEDT** **NXCAIHAKRV** **TIMPKDIQLA**  
**RRIRGERA**

so that the transcription of certain nuclear and mitochondrial genes may be coordinated. Additionally, a histone deacetylase, SirT3, is known to be transported to mitochondria during cell stress (Scher et al., 2007), suggestive that its histone substrate, H3, might also be dual-targeted to the mitochondria.

## 2. Materials and methods

### 2.1. Organelle isolation by differential centrifugation

Tissues were homogenized with cold mortar and pestle in 0.3 M mannitol, 0.02 M phosphate, pH 7.2 and Halt Protease Inhibitor Cocktail (Pierce kit #78410). Homogenate was first filtered through cheesecloth, then centrifuged to pellet nuclei and any intact cells, at 2000 *g* for 5 min at 4°C, and the pellets were resuspended in 2 ml 0.3 M mannitol, 0.02 M phosphate, pH 7.2; the remaining supernatant was then centrifuged to pellet any remaining nuclei; 2000 *g*, 5 min, 4°C; this step was performed to minimize nuclear contamination of the mitochondrial fraction. From the resulting supernatant, mitochondria were pelleted by centrifugation at 6000 *g*, 10 min, 4°C; and the pellet was resuspended in 2 ml 0.3 M mannitol, 0.02 M phosphate, pH 7.2. At least three separate organelle preparations were performed in this way, and all were independently subjected to the battery of Western blots described below.

### 2.2. Assay for mitochondrial succinate dehydrogenase activity

To test the quality of the final mitochondrial fraction, a modified succinate dehydrogenase assay was performed (Reisch and Elpeleg, 2007) to check for cellular respiration. The total protein content of the mitochondrial suspension was determined by Bradford Assay (see below), and three doses of mitochondrial protein were used for the assay: 58 µg/ml, high dose; 43.5 µg/ml,

medium dose and 29 µg/ml, low dose. The assay was performed at room temperature in 0.3 M mannitol, 0.02 M phosphate, 0.01 M KCl, 0.005 M MgCl<sub>2</sub>, 0.05 mM DCIP (2,6-dichlorophenol-indophenol), 0.02 M succinate, 40 mM NaN<sub>3</sub>, pH 7.2. As a control, the low dose of mitochondria was also run together with the competitive inhibitor malonate, 8 mM. Absorbance was measured at 600 nm every 5 min for 30 min, and change in absorbance for each time point was calculated by subtracting the absorbance from the original absorbance. In an additional control, omission of NaN<sub>3</sub> produced comparable data with the addition of malonate (data not shown). Assays were performed in triplicate from independent mitochondrial preparations.

### 2.3. SDS/PAGE and Western blot

Protein concentrations for nuclei and mitochondria fractions were determined by the Bradford Assay (Pierce 1856209). From each fraction, volumes containing equal amounts of protein were boiled for 5 min in sample buffer (Tris, SDS, 2-mercaptoethanol, pH 6.8) and run on 4–20% gradient SDS/polyacrylamide gels at 150 V for 1.5 h. A mixture of dual-colour (Bio-Rad 161-0374) and unstained (Bio-Rad 161-0363) molecular mass markers were used. Gels were stained with Coomassie G250 or transferred to nitrocellulose membranes (0.45 µm) by electrophoresis for 1 h at 400 mA. Membranes were blocked with 0.5% BSA, 0.1% Tween 20 in PBS, probed with various primary antibodies, as indicated below; amplified and HRP (horseradish peroxidase)-labelled with secondary antibody, peroxidase-conjugated goat-anti rabbit (Sigma A6667) or goat anti-mouse (Sigma A4416) and developed using a CN/DAB Substrate Kit (Pierce 34000) or Opti-4CN (Bio-Rad 170-8235). In Figures 2(A) and 2(C), the primary antibody was rabbit polyclonal IgG from Sigma (H 0164) against a synthetic C-terminal peptide of H3 (residues 125–136). A different C-terminal-specific H3 antibody, Active Motif 39164, produced identical results with those in Figures 2(A) and 2(C) (Supplementary Figure S1 at <http://www.cellbiolint.org/cbi/034/cbi0340997add.htm>). In Figures 2(B) and 2(D), the primary antibody was mouse monoclonal IgG1 from Abcam (ab4566) against fibrillarin, a nucleolar marker (Ochs et al., 1985; Reichow et al., 2007). In Figure 5(A), the primary antibody was rabbit polyclonal IgG from Abcam (ab18521) against a synthetic N-terminal peptide (residues 1–100) of H3; specificity was confirmed previously (Kurtz et al., 2009). In Figure 5(B), the primary antibody was rabbit polyclonal IgG from Abcam (ab39655) against an N-terminal peptide of H3. To probe unstained markers, Strept-tactin-HRP (Bio-Rad 161-0380) was added with the secondary antibody in some experiments. Gels and blots were digitally scanned

**Table 2 Cellular localization predictions for H3 as determined by WoLF PSORT and MitoProt II**

Species	Primary accession number of H3	Common name	WoLF PSORT score for nuclear localization sequence	WoLF PSORT score for mitochondrial targeting signal	MitoProt II probability of export to mitochondria
<i>Candida albicans</i>	Q59VN2	Yeast histone H3.1	0.97	0.25	0.9975
<i>Brassica napus</i>	Q6LCK1	Rape (plant) histone H3.2	0.97	0.87	0.9972
<i>Caenorhabditis elegans</i>	Q10453	Nematode histone H3.3	0.97	1.17	0.9985
<i>Drosophila melanogaster</i>	P84249	Fruit fly H3.3	0.97	1.17	0.9985
<i>Xenopus laevis</i>	Q92133	African clawed frog histone H3	0.97	0.87	0.9971
<i>Homo sapiens</i>	Q71DI3	Human histone H3	0.97	0.87	0.9973

with HP Scanjet G3010, and bands were analysed for molecular mass with UN-SCAN-IT gel software (Silk Scientific). Control blots probed with secondary antibody alone were negative (data not shown). Western blot data were confirmed in triplicate, from independent organelle preparations.

#### 2.4. Confirmation of antibody specificity through LC-MS/MS (liquid chromatography tandem mass spectrometry) protein identification

Using UN-SCAN-IT gel software, the protein band containing H3 from a Coomassie G250-stained gel of mitochondrial extract was chosen (Figure 3). The correct band was determined by comparison of its molecular mass with that of the Western-blotted mitochondrial H3 band. The bands were cut from the SDS/PAGE gel and submitted to The Rockefeller University Proteomics Resource Center for LC-MS/MS protein identification (Fernandez et al., 1998) (<http://pdtc.rockefeller.edu/>). H3 was positively identified in the sample. Other contaminating proteins were also identified in the submitted gel band.

#### 2.5. Subcellular localization prediction with WoLF PSORT and MitoProt

Protein sequences were taken from the ExpASY Proteomics Server, UniProtKB/Swiss-Prot database (<http://www.expasy.org/>). Subcellular localization was analysed with WoLF PSORT (Horton et al., 2007) (<http://wolfpsort.seq.cbr.crc.jp/>) and MitoProt (Claros and Vincens, 1996) (<http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html>).

#### 2.6. Subcellular localization with immunohistochemistry

Immunohistochemical stains and images were provided by HistoTox Labs, Inc. Fresh cauliflower floral meristem buds were dehydrated, cut into 4  $\mu\text{m}$  serial sections, nuclei were stained with haematoxylin (Dako, Cat. no. K8018), the sections were incubated in primary antibody solutions, signals were amplified with Envision+ Rabbit HRP linker (Dako K4010), and colour was developed with DAB+ Chromagen and hydrogen peroxide. All images were taken with a Leica DMLB microscope using a  $\times 63$  objective and an Olympus E410 camera. The primary antibodies were against the mitochondrial marker COX IV (Abcam, Cat. no. 160560) and against the C-terminus of H3 (Sigma, Cat. no. H0164). In the negative control, sections were treated in exactly the same way except for the omission of primary antibody. Envision+ Rabbit HRP linker (Dako, Cat. no. K4010) and DAB+ Chromagen (Dako, Cat. no. K3468) were used for immunogenic colour development.

### 3. Results

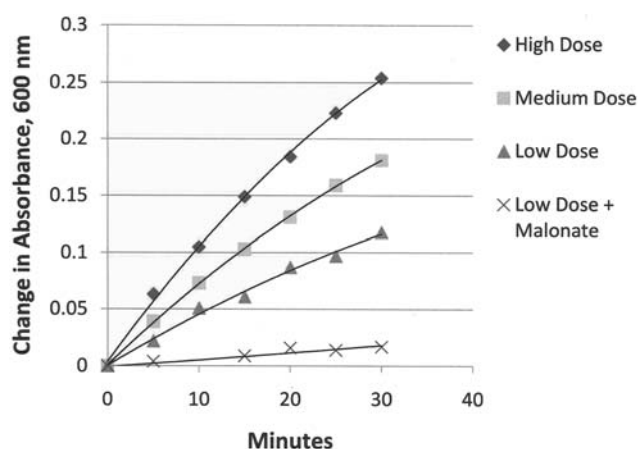
#### 3.1. Mitochondrial enrichment

The enrichment of mitochondria in the mitochondrial fraction was confirmed by a modified succinate dehydrogenase assay for

cellular respiration (Reisch and Elpeleg, 2007). The enzyme activity of the mitochondrial fraction was dose dependant and was inhibited by the addition of malonate (Figure 1) and by the omission of sodium azide (data not shown).

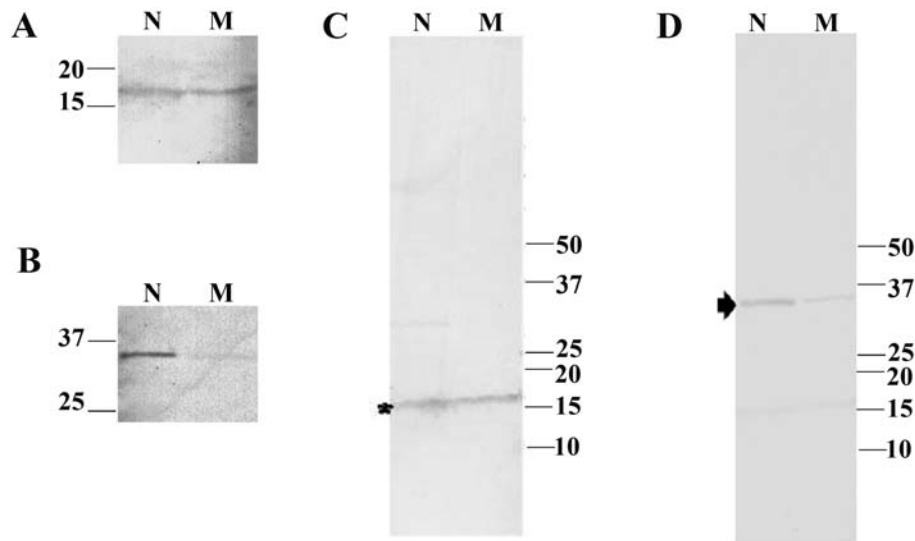
#### 3.2. H3 in the mitochondria

Positive Western blots for mitochondrial H3 were achieved using a commercial antibody (Sigma H0164) to the highly conserved C-terminus of the protein (Figure 2A). A different C-terminal-specific H3 antibody, Active Motif 39164, produced identical results to those in Figures 2(A) and 2(C) (Supplementary Figure S1). Use of C-terminus-specific antibodies to probe H3 allowed us to avoid the N-terminal mitochondrial targeting sequence, which may be removed or modified in the mitochondria. All controls using secondary antibody alone were negative (data not shown). As shown in Figures 2(A) and 2(C), the strength of the signal for H3 in the mitochondrial fraction was almost equal to that of the nuclear fraction. The strength of the signal indicates that H3 is present at a high concentration in the mitochondrial fraction and that strong presence of H3 cannot be explained by the minimal degree of nuclear contamination. The degree of nuclear contamination of the mitochondrial fractions was determined by Western blots performed with an antibody against the nucleolar marker, fibrillarin. The fibrillarin signal was strong in the nuclear fraction but very weak in the mitochondrial fraction (Figure 2B). To further illustrate this point, Western blots that had first been probed for H3 with H0164 were digitally scanned, and then the same blots were re-probed with anti-fibrillarin (Figures 2C and 2D). Omission of protease inhibitors during mitochondrial isolation had no impact on the Western blot data (data not shown). This indicates that mitochondrial H3 is located inside the organelle, where it is protected from lysosomal enzyme degradation, as opposed to being attached to the cytoplasmic surface of the mitochondrial membrane.



**Figure 1** Succinate dehydrogenase assay for mitochondrial activity

Three doses of mitochondrial protein were used as a source of succinate dehydrogenase; 58  $\mu\text{g}/\text{ml}$ , high dose; 43.5  $\mu\text{g}/\text{ml}$ , medium dose and 29  $\mu\text{g}/\text{ml}$ , low dose; with DCIP colour change as an indicator of enzyme activity. As a control, the low dose of mitochondria was also run together with the competitive inhibitor, malonate. In an additional control, omission of  $\text{Na}_3\text{N}$  produced comparable data with the addition of malonate (data not shown).



**Figure 2** Presence of H3 in mitochondria and confirmation that mitochondrial fraction was mostly free of nuclei  
Western blots of nuclear (N) and mitochondrial (M) extracts from *B. oleracea*: (A) probed with Sigma H0164 to the conserved C-terminus of H3; (B) probed with Abcam ab4566, an antibody to the nucleolar marker fibrillarlin. In (C), a blot was probed first with Sigma H0164 to H3, (marked \*), and in (D), the same blot from (C) was re-probed with Abcam ab4566, to fibrillarlin, marked with arrowhead. A different C-terminal-specific H3 antibody, Active Motif 39164, produced identical results to those in Figures 2(A) and 2(C) (see Supplementary Figure S1 at <http://www.cellbiolint.org/cbi/034/cbi034xxxadd.htm>). Molecular masses of standards are indicated in kDa.

Specificity of the H0164 antibody to H3 in the mitochondria was confirmed by LC-MS/MS verification of the identity of the positive protein in the mitochondria fraction as H3. The corresponding gel band (Figure 3), as determined by molecular mass comparison to Western blot data with UN-SCAN-IT gel software, was cut from a Coomassie-stained gel and submitted to The Rockefeller University Protein/DNA Technology Center (Fernandez et al., 1998). Their analysis resulted in a match to H3 (accession number Q43566) with sequence coverage of 37% (Table 1). Other contaminating proteins were also identified in the sample, as expected from a crude mitochondrial preparation containing the entire mitochondrial proteome (Figure 3 and Supplementary Table S1 at <http://www.cellbiolint.org/cbi/034/cbi0340997add.htm>), and as further evidence that nuclear contamination was minimal in the mitochondrial fraction, none of the other proteins identified by LC-MS/MS are solely localized to the nucleus, with the exception of histone H2A (future studies will be needed to explore the possibility that histone H2A may also be targeted to the M).

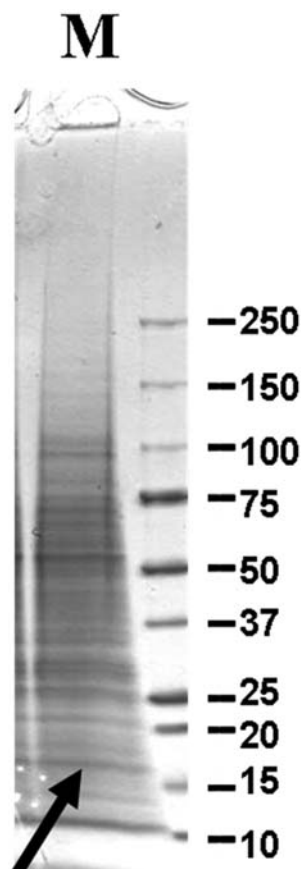
Immunohistochemical staining of fresh cauliflower floral meristem buds was performed to look for the presence of H3 in the mitochondria and in the nucleus (Figure 4). Strong positive staining for H3 was seen in the nucleus, as expected, and positive staining was also seen in an extranuclear distribution that resembled the mitochondrial staining obtained by probing serial sections for the mitochondrial marker COX IV. Nuclei were first stained with haematoxylin (Figures 4A, 4B and 4C), then the mitochondria were probed with an antibody to COX IV in some sections (Figure 4A) or with an antibody against the conserved C-terminus of H3 (Figure 4B) or as a negative control, all primary antibody were omitted (Figure 4C). The extranuclear H3 staining pattern in Figure 4(B) resembles the COX IV staining in the serial section in Figure 4(A), suggesting the presence

of H3 in the mitochondria. Immunohistochemical stains and images were provided by HistoTox Labs Inc.

### 3.3. Mitochondrial targeting of H3 and evidence of unique N-terminal modification of mitochondrial H3 compared with nuclear H3

As shown in Table 2, WoLF PSORT localization software scored the mitochondrial targeting signal of H3 with values that were comparable, and sometimes higher, than its nuclear localization sequence scores. Using a second software application, MitoProt II, we confirmed that the probability of H3 export to M was very high.

Typically the N-terminal targeting signal is removed from proteins after they are transported into the mitochondria, but that removal is not required for import (Glaser et al., 1998). Although WoLF PSORT predicted a cleavage site at the 45th residue, Western blots with primary antibody to the C-terminus of H3 did not demonstrate any loss of molecular mass by mitochondrial H3 (Figures 2A and 2C). Interestingly, Western blots performed with two different antibodies against the N-terminus of H3 (Kurtz et al., 2009) produced a strong H3 signal in the nucleus, but only a very weak signal in the mitochondria, indicating that the N-terminus of mitochondrial H3 was modified compared with nuclear H3 (Figure 5). By comparison with the H3 signal attained with the C-terminal-specific antibody (Figure 2A), we learned that mitochondrial H3 is different from nuclear H3, and the difference in reactivity of H3 with C-terminal-specific and N-terminal-specific antibodies further strengthens our position that the strong H3 signal seen with the C-terminal antibody was not due to nuclear contamination of the mitochondria, but rather, H3 is a dual-targeted protein whose N terminus is modified in the mitochondria.

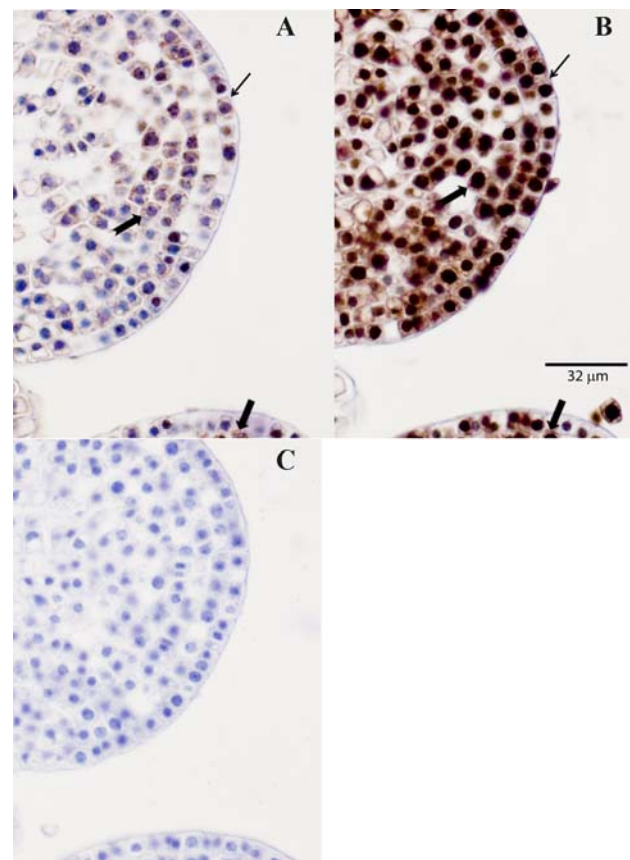


**Figure 3** Coomassie G250-stained SDS/PAGE of mitochondrial extract (M). Arrow at the bottom of the figure indicates the band selected for submission to The Rockefeller University Proteomics Resource Center. Molecular mass markers are indicated on the right.

## 4. Discussion

Future studies will aim at understanding the relationship between dual-targeted transcription factors and dual-targeted H3 and how mitochondrial H3 may contribute to the co-ordination of metabolism, transcription, replication and even apoptosis between the mitochondria and the nucleus. Study of mitochondrial H3 may also provide clues as to how eukaryotes evolved from prokaryotes. According to the endosymbiotic theory, eukaryotic cells acquired mitochondria and plastids from mutualistic relationships with ancient prokaryotic organisms, hence the presence of bacteria-like DNA and ribosomes in the mitochondria (Gray and Doolittle, 1982).

During the evolution of each eukaryotic species, mitochondria lose large portions of the original mitochondrial genome. Although modern mitochondrial genes encode some of the mitochondrial proteins, most of these proteins are targeted to mitochondria after being expressed by nuclear genes. Nuclear genes that encode mitochondrial proteins are thought to have transferred to the nucleus from the mitochondria at one point and later gained mitochondrial targeting sequences by poorly understood mechanisms (Adams and Palmer, 2003). Transferred genes can be identified by their homology to the DNA of those prokaryotes thought to be related to the ancestors of the mitochondria. Indeed,

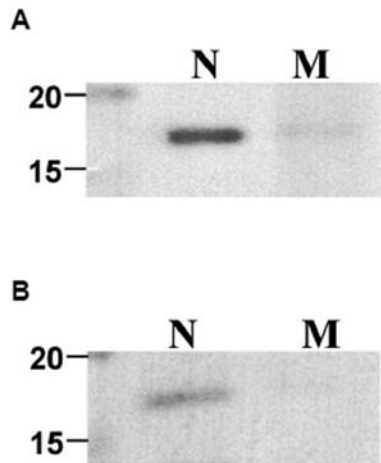


**Figure 4** Immunohistochemical staining of mitochondria in fresh cauliflower floral meristem buds, 4  $\mu\text{m}$  serial sections

Nuclei were stained in (A), (B) and (C) with haematoxylin (Dako, Cat. no. K8018). In (A), the primary antibody was against the mitochondrial marker COX IV (Abcam, Cat. no. 160560). In (B), the primary antibody was against the C-terminus of H3 (Sigma, Cat. no. H0164). The negative control in (C) was treated exactly like (A) and (B) except for the omission of primary antibody. Envision+ Rabbit HRP linker (Dako, Cat. no. K4010) and DAB+ Chromagen (Dako, Cat. no. K3468) were used for immunogenic colour development (immunohistochemical stains and images provided by HistoTox Labs Inc.).

one report lists a gene for H3 among the many genes that may have originated from the mitochondrial genome (Elo et al., 2003), and a recent quantitative liquid chromatography-mass spectroscopy study of the mitochondrial proteome of rat revealed H3 in the mitochondria of these mammalian cells (Johnson et al., 2007).

In conclusion, we report the dual-targeting of H3 to the mitochondria and to the nucleus in *B. oleracea*. WoLF PSORT and MitoProt II predicted that H3 is similarly targeted in most eukaryotic species. Traditionally, H3 has been known to target to the nucleus exclusively; so to minimize the possibility that our results were due to nuclear contamination of the mitochondrial fraction, we have used three strategies. First, although the differential centrifugation technique we utilized does not produce completely pure organelle fractions, the sedimentation properties of nuclei and of mitochondria are quite different, and the second centrifugation step was performed to help minimize nuclear contamination. The level of nuclear contamination was tested and confirmed to be low using an antibody to the nucleolar marker fibrillarin. Secondly, if the H3 in the mitochondria were simply an artefact of nuclear contamination, then we would expect all antibodies that recognize



**Figure 5** Western blot evidence that the amino terminus of mitochondrial H3 is different from that of nuclear H3

Nuclear (N) and mitochondrial (M) extracts from *B. oleracea* probed with (A) Abcam ab18521, an antibody to the N-terminal of H3, amino acids 1–100 and (B) Abcam ab39655, an antibody to another N-terminal synthetic peptide. Molecular masses of standards are indicated on the left in kDa.

nuclear H3 to also recognize the H3 in the mitochondria; however, antibodies against the N-terminus recognize nuclear H3, but not mitochondrial H3. Thirdly, immunohistochemistry confirmed the presence of H3 outside of the nucleus. We believe that these three lines of data provide adequate evidence that the H3 identified in the mitochondria is not due to nuclear contamination of the mitochondrial fraction. We predict that the study of mitochondrial H3 will aid in our understanding of cellular coordination between the genes of the nucleus and those of the mitochondrion and will reveal new knowledge about the course of the evolution of modern mitochondrial genomes and proteomes.

#### Author contribution

Mary Katherine Zanin was the primary investigator. She was responsible for overseeing all projects in concept, design, data collection and analysis. She drafted 84% of the manuscript and was the primary editor of the manuscript. Jon Donohue made significant contributions to the design, data collection and analysis for the samples sent to Rockefeller University for LC-MS/MS protein identification. He also made minor contributions to the manuscript, 8%, and approved the final version to be published. Bryan Everitt made significant contributions to the design and analysis of Western blot experiments. He also made minor contributions to the manuscript, 8%, and approved the final version to be published.

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## References

Adams KL, Palmer JD. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol Phylogenet Evol* 2003;29:380–95.

- Agalioti T, Lomvardas S, Parekh B, Yie J, Maniatis T, Thanos D. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. *Cell* 2000;103:667–8.
- Alevizopoulos A, Dusserre Y, Tsai-Pflugfelder M, von der Weid T, Wahli W, Mermoud N. A proline-rich TGF-beta-responsive transcriptional activator interacts with histone H3. *Genes Dev* 1995;9:3051–66.
- Bartova E, Krejci J, Harnicarova A, Galiova G, Kozubek S. Histone modifications and nuclear architecture: a review. *J Histochem Cytochem* 2008;56:711–21.
- Binder S, Brennicke A. Gene expression in plant mitochondria: transcriptional and post-transcriptional control. *Philos Trans R Soc Lond B Biol Sci* 2003;358:181–8.
- Caron F, Jacq C, Rouviere-Yaniv J. Characterization of a histone-like protein extracted from yeast mitochondria. *Proc Natl Acad Sci U S A* 1979;76:4265–9.
- Claros MG, Vincens P. Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur J Biochem* 1996;241:779–86.
- Dement GA, Treff NR, Magnuson NS, Franceschi V, Reeves R. Dynamic mitochondrial localization of nuclear transcription factor HMGA1. *Exp Cell Res* 2005;307:388–401.
- Dilworth FJ, Fromental-Ramain C, Yamamoto K, Chambon P. ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR *in vitro*. *Mol Cell* 2000;6:1049–58.
- Elo A, Lyznik A, Gonzalez DO, Kachman SD, Mackenzie SA. Nuclear genes that encode mitochondrial proteins for DNA and RNA metabolism are clustered in the *Arabidopsis* genome. *Plant Cell* 2003;15:1619–31.
- Fernandez J, Gharahdaghi F, Mische SM. Routine identification of proteins from sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels or polyvinyl difluoride membranes using matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS). *Electrophoresis* 1998;19:1036–45.
- Garrido N, Griparic L, Jokitalo E, Wartiovaara J, van der Bliek AM, Spelbrink JN. Composition and dynamics of human mitochondrial nucleoids. *Mol Biol Cell* 2003;14:1583–96.
- Glaser E, Sjoling S, Tanudji M, Whelan J. Mitochondrial protein import in plants. Signals, sorting, targeting, processing and regulation. *Plant Mol Biol* 1998;38:311–38.
- Gray MW, Doolittle WF. Has the endosymbiont hypothesis been proven? *Microbiol Rev* 1982;46:1–42.
- Hatzis P, Talianidis I. Dynamics of enhancer-promoter communication during differentiation-induced gene activation. *Mol Cell* 2002;10:1467–77.
- Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams-Collier CJ et al. WoLF PSORT: protein localization predictor. *Nucleic Acids Res* 2007;35:W585–7.
- Johnson DT, Harris RA, French S, Blair PV, You J, Bemis KG et al. Tissue heterogeneity of the mammalian mitochondrial proteome. *Am J Physiol Cell Physiol* 2007;292:C689–97.
- Kaufman BA, Newman SM, Hallberg RL, Slaughter CA, Perlman PS, Butow RA. In organello formaldehyde crosslinking of proteins to mtDNA: identification of bifunctional proteins. *Proc Natl Acad Sci U S A* 2000;97:7772–7.
- Krause K, Kilbiński I, Mulisch M, Rodiger A, Schafer A, Krupinska K. DNA-binding proteins of the Whirly family in *Arabidopsis thaliana* are targeted to the organelles. *FEBS Lett* 2005;579:3707–12.
- Kurtz K, Ausio J, Chiva M. Preliminary study of sperm chromatin characteristics of the brachyuran crab *Maja brachydactyla*. Histones and nucleosome-like structures in decapod crustacean sperm nuclei previously described without SNBPs. *Tissue Cell* 2009;41:334–44.
- Kutsyi MP, Gouliava NA, Kuznetsova EA, Gaziev AI. DNA-binding proteins of mammalian mitochondria. *Mitochondrion* 2005;5:35–44.
- Newman SM, Zelenaya-Troitskaya O, Perlman PS, Butow RA. Analysis of mitochondrial DNA nucleoids in wild-type and a mutant strain of *Saccharomyces cerevisiae* that lacks the mitochondrial HMG box protein Abf2p. *Nucleic Acids Res* 1996;24:386–93.

- Nguyen TT, Cho K, Stratton SA, Barton MC. Transcription factor interactions and chromatin modifications associated with p53-mediated, developmental repression of the alpha-fetoprotein gene. *Mol Cell Biol* 2005;25:2147–57.
- Ochs RL, Lischwe MA, Spohn WH, Busch H. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. *Biol Cell* 1985;54:123–33.
- Parrizas M, Maestro MA, Boj SF, Paniagua A, Casamitjana R, Gomis R et al. Hepatic nuclear factor 1-alpha directs nucleosomal hyperacetylation to its tissue-specific transcriptional targets. *Mol Cell Biol* 2001;21:3234–43.
- Reichow SL, Hama T, Ferre-D'Amare AR, Varani G. The structure and function of small nucleolar ribonucleoproteins. *Nucleic Acids Res* 2007;35:1452–64.
- Reisch AS, Elpeleg O. Biochemical assays for mitochondrial activity: assays of TCA cycle enzymes and PDHc. *Methods Cell Biol* 2007;80:199–222.
- Salganik RI, Dudareva NA, Kiseleva EV. Structural organization and transcription of plant mitochondrial and chloroplast genomes. *Electron Microsc Rev* 1991;4:221–47.
- Scher MB, Vaquero A, Reinberg D. SirT3 is a nuclear NAD<sup>+</sup>-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. *Genes Dev* 2007;21:920–8.
- Schwacke R, Fischer K, Ketelsen B, Krupinska K, Krause K. Comparative survey of plastid and mitochondrial targeting properties of transcription factors in *Arabidopsis* and rice. *Mol Genet Genomics* 2007;277:631–46.
- Soutoglou E, Talianidis I. Coordination of PIC assembly and chromatin remodeling during differentiation-induced gene activation. *Science* 2002;295:1901–4.
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000;403:41–5.

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