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Note

An Efficient Genetic Screen in Drosophila to Identify Nuclear-Encoded Genes With Mitochondrial Function

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ABSTRACT

We conducted a screen for glossy-eye flies that fail to incorporate BrdU in the third larval instar eye disc but exhibit normal neuronal differentiation and isolated 23 complementation groups of mutants. These same phenotypes were previously seen in mutants for *cytochrome c oxidase subunit Va*. We have molecularly characterized six complementation groups and, surprisingly, each encodes a mitochondrial protein. Therefore, we believe our screen to be an efficient method for identifying genes with mitochondrial function.

MITOCHONDRIAL function is essential for a number of important cellular processes, such as the generation of ATP (reviewed in Ackerman and Tzagoloff 2005), apoptosis (reviewed in Newmeyer and Ferguson-Miller 2003), and the regulation of aging (reviewed in Ohta 2003). Recently, work from our laboratory has shown that the metabolic status of a cell, controlled by mitochondrial function, also regulates the G_1–S checkpoint in mitosis (Mandal *et al.* 2005). This is evident in *tenured* (*tend*) mutants, which contain a null mutation in the gene encoding cytochrome *c* oxidase subunit Va of complex IV of the mitochondrial electron transport chain. This mutation causes a reduction in ATP generated in mutant cells to ~40% of wild-type levels, triggering the activation of AMPK and p53, which leads to the eventual down-regulation of cyclin E. A G_1–S mitotic checkpoint is then enforced, preventing cells from reentering the cell cycle (Mandal *et al.* 2005).

Adult *tend* mutants have glossy eyes lacking lens material, normally secreted by cone and pigment cells of the eye. In earlier studies, the lens-depleted glossy-eye phenotype was described in *lozenge* mutants, which are defective in cone and pigment cell specification (Flores *et al.* 1998). However, in the case of *tend*, such defects are caused by the lack of a sufficient number of precursor cells from which cone and pigment cells arise (Mandal *et al.* 2005).

The events that give rise to the *tend* adult eye phenotype occur in larval eye development. The larval Drosophila eye imaginal disc undergoes two distinct phases of proliferation (Wolff and Ready 1991). During the early larval stages prior to differentiation, all cells divide frequently and asynchronously, apparently without regulation. In the third larval instar, the morphogenetic furrow sweeps across the eye disc to pattern the cells (Ready *et al.* 1976). Within the morphogenetic furrow, cells arrest in G_1 and, following the passage of the furrow, either differentiate into neuronal photoreceptors or reenter the cell cycle and undergo a terminal round of division termed the *s*econd *m*itotic *w*ave (SMW). This generates the precursors for cone and pigment cell lineages (Wolff and Ready 1991). Misregulation of the SMW will lead to a loss of these accessory cell types. In the third larval instar, cells mutant for *tend* anterior to the furrow slow down in cell cycle progression, while those posterior to the furrow fail to cross the G_1–S checkpoint due to impaired ATP production (Mandal *et al.* 2005). Therefore, in addition to undergoing fewer rounds of mitosis prior to the passage of the furrow, *tend* mutants lack the SMW and, as a result, have a dramatically reduced number of accessory cells. Importantly, in *tend* mutant cells, cell

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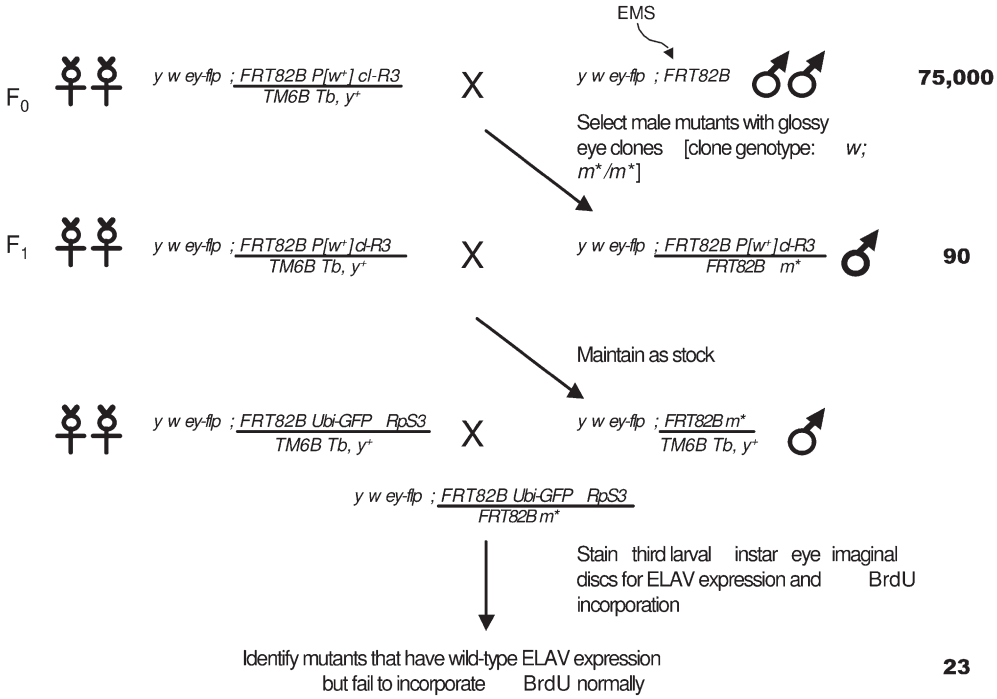

Figure 1.—Crossing scheme for genetic screen. A total of 75,000 chromosomes were mutagenized and screened for the adult glossy-eye phenotype. A total of 90 glossy-eye mutants were isolated, and larval eye-disc clones were induced to study their BrdU incorporation and ELAV expression. Twenty-three failed to incorporate BrdU but expressed ELAV normally and were studied further. *m** represents the EMS-induced mutation. The *cl-R3* mutation is cell lethal and is used to eliminate cells that are homozygous for the chromosome that does not contain *m**. Similarly, for larval clones, cells homozygous for the *RpS3* (*Minute*) mutation are eliminated. Cells heterozygous for the *cl-R3* and *RpS3* mutations grow more slowly, allowing *m*/m** cells to form large clones.

divisions in second and early third instar eye discs are not affected. As a result, *tend* mutants give rise to large clones in the adult eye. This is different from the phenotype resulting from mutant clones in the basic cell cycle machinery, which would not be able to give rise to any mutant clones. Furthermore, early patterning and differentiation of neurons are also not affected in *tend*, as mutant clones express pan-neuronal, as well as cell-type-specific, markers and extend axons normally to the optic lobe (Mandal *et al.* 2005).

On the basis of the nature of the *tend* phenotype, we hypothesized that a simple screen that identifies glossy-eye flies, followed by a secondary screen for a subset of the mutants in which cells exhibit proliferation defects but have relatively normal patterning in the third larval instar, will potentially identify nuclear genes that encode mitochondrial proteins. Our hypothesis is supported by our earlier finding that, like *tend*, flies mutant in the mitochondrial components *pdsw*, *mRpL4*, and *mRpL17* exhibit a glossy-eye phenotype and fail to incorporate BrdU in the larval eye disc but stain normally with the ELAV antibody (Mandal *et al.* 2005). In this article, we show that an unbiased screen for glossy-eye mutants that exhibit these larval phenotypes is remarkably effective in identifying genes with mitochondrial function.

We conducted an *eyeless-flp/FRT*-based mitotic recombination screen (Newsome *et al.* 2000) on chromosome arm 3R (Figure 1). *y w ey-flp; FRT 82B* males were mutagenized with 25 mm ethyl methanesulfonate (EMS) and crossed to *y w ey-flp; FRT82B P[w+] cl-R3/TM6B Tb, y+* females to generate adult eye clones. *ey-flp* drives mitotic recombination between the FRT-containing chromosomes under the control of the *eyeless* enhancer, specifically in the embryonic anlagen of the developing eye (Newsome *et al.* 2000). The resulting clones of cells will be homozygous for the newly induced mutation and are white due to the lack of the w^+ gene essential for pigmentation. A total of 75,000 adult flies were screened for the mosaic glossy-eye phenotype. Ninety mutants were isolated. Complementation analysis was then performed by crossing each of the 90 mutants with one another and looking for lethality in the offspring. This yielded a total of 46 complementation groups. All mutants were balanced over a *TM6B Tb,* y^+ chromosome and maintained as stocks.

Next, third larval instar eye imaginal discs from the identified glossy-eye mutants were assayed for BrdU incorporation and stained with the neuron-specific ELAV antibody (Yao *et al.* 1993). Larval eye-disc clones were generated by crossing the mutant stocks to *y w ey-flp; FRT82B Ubi-GFP RpS3/TM6B Tb,* y^+ flies. Clones homozygous for the mutation are negatively marked by their lack of GFP. The 46 complementation groups isolated from the glossy-eye screen fall into four categories on the basis of this characterization. Ten mutant groups exhibit both wild-type neuronal patterning and BrdU incorporation, indicating that the glossy-eye phenotype is due to mutations in genes that function after the third instar and are required for cone and pigment cell specification. Three mutant groups display abnormal ELAV staining patterns while cells incorporate BrdU normally. Since the formation of cone cells requires a combination of signals from differentiated neuronal photoreceptors (Flores *et al.* 2000), this phenotype likely occurs because of defects in the patterning

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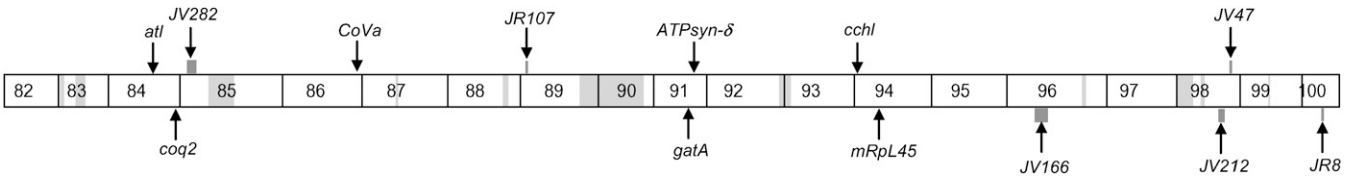
TABLE 1

Mapping results and mutations in nuclear-encoded genes with mitochondrial function that exhibit a G_1–S block in the third larval instar with normal neuronal differentiation

A. Mapping results



Cytological position	Gene name	Mitochondrial process	Alleles
	B. Mitochondrial genes identified by the glossy eye–BrdU screen		
84E4	*CG10092 arginyl tRNA ligase* (*atl*)	Protein biosynthesis–tRNA synthesis	*JR70, JR77, JV83, JR101, JR203, JV219, JR247, JV250, JP782*
84F11	*CG9613 coenzyme Q biosynthesis protein 2* (*coq2*)	Oxidative phosphorylation–complex III	*JV259, JP768*
91E4	*glutamyl-tRNA amidotransferase A* (*gatA*)	Protein biosynthesis–tRNA synthesis	*JR15, JV87, JR94, JR113, JR205*
91F1	*ATP synthase subunit* δ (*ATPsyn-*δ)	Oxidative phosphorylation–complex V of electron transport chain	*JR92, JV115, JR238*
94A1	*CG6022 cytochrome c heme lyase* (*cchl*)	Oxidative phosphorylation–complex IV of electron transport chain	*JV32, JV56, JV193, JR214*; Exelixis *c04553* (Thibault *et al.* 2004)
94B6	*mitochondrial ribosomal protein large subunit 45* (*mRpL45*)	Protein biosynthesis–ribosome assembly	*JV28*
	Previously identified mitochondrial genes that cause the glossy-eye–defective BrdU incorporation phenotype		
23F3	*pdsw*	Oxidative phosphorylation–complex I of electron transport chain	$pdsw^{k10101}$ (Spradling *et al.* 1999)
35F11	*mitochondrial ribosomal protein large subunit 4* (*mRpL4*)	Protein biosynthesis–ribosome assembly	$mRpL4^{k14608}$ (Spradling *et al.* 1999)
61B3	*mitochondrial ribosomal protein large subunit 17* (*mRpL17*)	Protein biosynthesis–ribosome assembly	$mRpL17^{KG06809}$ (Bloomington Drosophila Stock Center at Indiana University)
86F9	*cytochrome c oxidase subunit Va (CoVa)*	Oxidative phosphorylation–complex IV of electron transport chain	*JP785* (Mandal *et al.* 2005)

Allele	Cytological position	No. of genes in region	Allele	Cytological position	No. of genes in region
		Other mapping results			
JV282	85A6-B1	24	*JV212*	98E1-E3	6
JR107	89A5-A8	29	*JV47*	98F5	9
JV166	96A21-B4	34	*JR8*	100B9	5

In A, dark shading indicates complementation groups that have mapped to a deficiency and light shading indicates gaps within the available deficiency kit. The candidate regions and potential mitochondrial genes for complementation groups that have not been cloned are also indicated.

of specific photoreceptors. Ten mutant groups exhibit defective ELAV staining patterns in early clusters as well as defects in BrdU incorporation, which can be attributed to mutations in genes required for specification of multiple cell types in the eye. Finally, 23 mutant groups fail to incorporate BrdU properly but show essentially normal patterning of neuronal clusters emerging from the furrow. They were further analyzed as part of this study.

We have mapped all mutants from this last category to either available deficiencies on chromosome 3R from the Bloomington and Exelexis collections (Thibault *et al.* 2004) or attributed their map positions to gaps within the deficiency kit (Table 1A). We have cloned

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six mutant complementation groups, consisting of 24 alleles, and obtained sequence information on the molecular lesions of 15 of the alleles (Figure 2). As the guiding hypothesis of this screen was to identify mitochondrial mutations, we sequenced potentially mitochondrial genes within candidate regions as determined through BLAST analysis (Altschul *et al.* 1990) and MitoProt scores, which predict potential mitochondrial localization on the basis of the presence of a mitochondrial localization sequence (Claros and Vincens 1996). Using this method, we identified four mutants in *CG6022*, a homolog of *cytochrome c heme lyase* (*cchl*); two mutant alleles of *CG9613*, a homolog of *coenzyme Q biosynthesis protein 2* (*coq2*); nine mutants in *CG10092*, a homolog of arginyl tRNA ligase (*atl*); three mutant alleles of *ATP synthase-δ subunit* (*ATPsyn-δ*); five mutants in *glutamyl-tRNA amidotransferase A* (*gatA*); and one mutant allele of *mitochondrial ribosomal protein large subunit 45* (*mRpL45*). Combined with previously described mutants in *pdsw*, *mRpL4*, *mRpL17*, and *tend*, all mutations exhibiting the glossy-eye phenotype with defective BrdU incorporation and wild-type neuronal patterning cloned thus far could be rationalized as having roles in the mitochondrion (see below and Figure 3 and Table 1B). Note that this screen identified the first mutant alleles for *CoVa*, *coq2*, *atl*, *ATPsyn-δ*, *gatA*, and *mRpL45*. Single alleles of *cchl*, *pdsw*, *mRpL4*, and *mRpL17* had been identified as lethal mutations in either the Exelixis collection or the Berkeley Drosophila Genome Gene Disruption Project, but were not previously genetically characterized (Spradling *et al.* 1999; Thibault *et al.* 2004) (Table 1B).

CCHL: Within the electron transport chain, CCHL participates in the function of complex IV by attaching a heme group to apocytochrome *c*, thereby converting it to a functional holocytochrome *c* and enabling its import through the inner membrane of the mitochondrion (reviewed in Kranz *et al.* 1998). In *Saccharomyces cerevisiae*, strains lacking or mutant in CCHL have increased levels of apocytochrome *c* in the cytoplasm (Dumont *et al.* 1991). From the screen, we have isolated four alleles of *cchl* (Figure 2) on the basis of their failure to complement Exelixis *c04553* (Thibault *et al.* 2004), which harbors a lethal insertion in *cchl*. The first allele, *JR214*, harbors a T > C transition resulting in the amino acid substitution M25T. *JV56* contains a C > T transition, resulting in a premature stop codon at Q65. The remaining two alleles, *JV193* and *JV32*, have G > A transitions at the first and last base pair of intron 2, respectively, and thus may result in the insertion of 19 amino acids if splicing cannot occur normally. All of the mutations occur within the heme lyase domain of the protein. Drosophila CCHL bears 53% identity to the human homolog and 42% identity to the *S. cerevisiae* homolog. According to MitoProt predictions, CCHL has a 28% likelihood of localizing to the mitochondrion; this relatively low likelihood may be due to the fact that mitochondrial heme lyases lack an N-terminal targeting signal and instead are directed to the mitochondrion by an internal localization sequence in the third quarter of the protein (Diekert *et al.* 1999). This is further supported by the fact that MitoProt also predicts a very low (4.8%) chance of mitochondrial localization for the *S. cerevisiae* CCHL.

ATPsyn-δ: Studies in *Escherichia coli* show that F_1F_0-ATP synthase, complex V of the electron transport chain, generates ATP through cooperation between a "rotor" and a "stator" (reviewed in Boyer 1997; Weber *et al.* 2004). The stator consists of two β-subunits and one δ-subunit (reviewed in Dunn *et al.* 2000), and loss of the δ-binding site on the α-subunit of F_1 results in impaired growth and ATP synthesis *in vivo* (Weber *et al.* 2004). Three alleles of *ATPsyn-δ* were isolated from the screen (Figure 2). *JV115* contains a G > A transition that results in a premature stop codon at W54, while *JR92* has a C > T transition that prematurely terminates the protein at Q83. A molecular lesion for the third allele, *JR238*, has not been found; presumably, it is located within a regulatory region of the gene. The mutations all lie within the ATP synthase-δ domain of the protein. MitoProt analysis suggests that Drosophila ATPsyn-δ has a 92% likelihood of localizing to the mitochondria.

COQ2: *coq2* encodes the enzyme parahydroxybenzoate (PHB):polyprenyltransferase (Ashby *et al.* 1992). As part of a multi-step process involved in ubiquinone biosynthesis, PHB:polyprenyltransferase catalyzes the transfer of a polyprenyl group from polyprenyl diphosphate to parahydroxybenzoate to form 3-polyprenyl-4-hydroxybenzoate (Winrow and Rudney 1969). Once synthesized, ubiquinone functions as an electron transporter between complexes I, II, and III of the electron transport chain (Green 1966). Two alleles of *coq2* were identified from the screen (Figure 2). The first allele, *JP768*, contains a 94-bp deletion mutation starting at A100 that causes a frameshift in the remaining sequence. All amino acids downstream of the deletion are altered, and a premature stop codon is introduced after the 232nd amino acid. *JV259* has a T > A transversion that results in a premature stop codon at L189. Both mutations fall within the UbiA prenyltransferase domain of COQ2. Drosophila COQ2 has 45% identity to the *S. cerevisiae* homolog and a 97% likelihood of being imported into the mitochondrion according to MitoProt analysis.

GatA: Aminoacylation studies have shown that there is no detectable glutaminyl–tRNA synthase activity in cyanobacteria, Gram-positive bacteria, and chloroplasts and mitochondria of plants and animals (Wilcox and Nirenberg 1968; Schon *et al.* 1988). Instead, these organisms and organelles synthesize glutamine by mischarging a $tRNA^{Gln}$ with glutamate and then using glutamyl–tRNA amidotransferases to convert the glutamate to glutamine (Schon *et al.* 1988). According to MitoProt predictions, Drosophila GatA has a relatively

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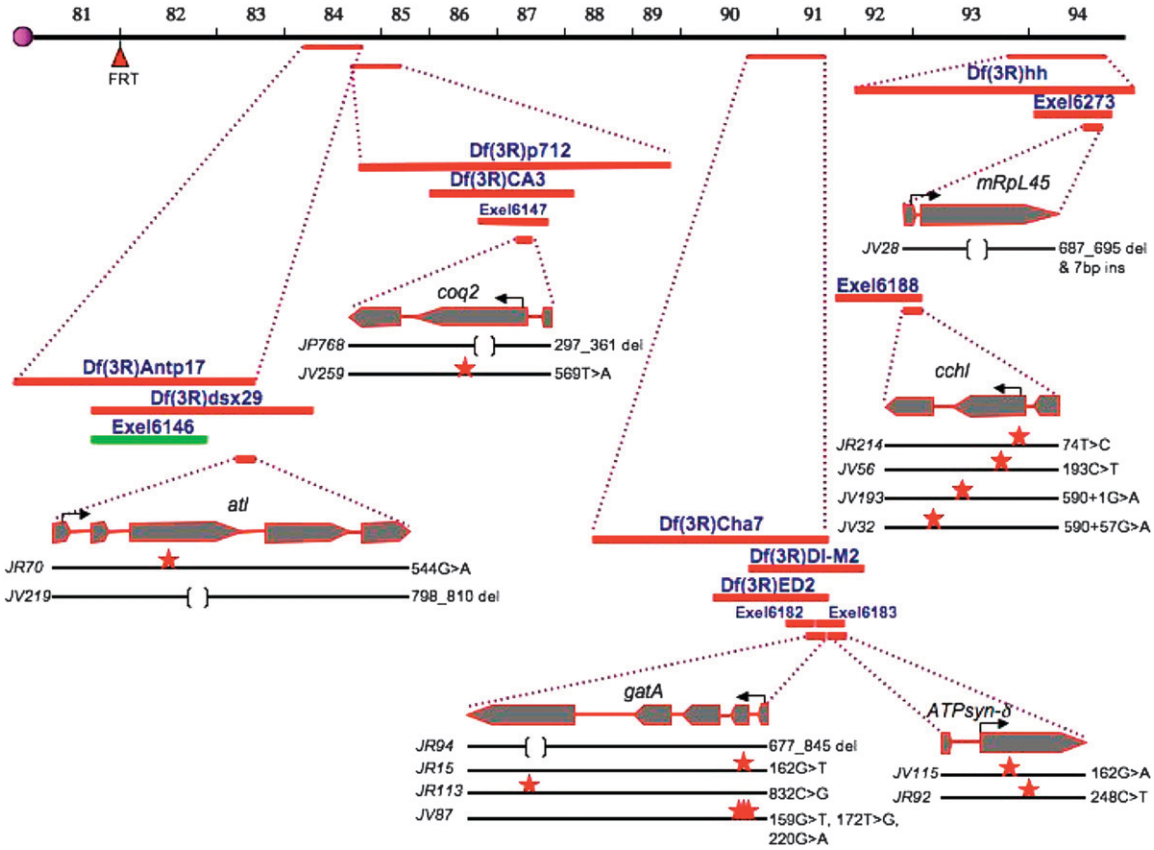

Figure 2.—Map positions and molecular lesions of identified mutations. Deficiency stock names and their approximate endpoints, as annotated by FlyBase, are indicated. Solid black lines, alleles identified from the screen; red stars, point mutations; brackets, deletions; red bars, deficiencies that fail to complement the mutant; green bars, deficiencies that complement the mutant.

low probability (56%) of localizing to the mitochondrion. To determine the exact subcellular location of GatA, we constructed a GatA–GFP fusion protein, transfected Drosophila S2 cells with the construct, and co-immunostained the cells with a GFP antibody and MitoTracker Orange CM-H2TMRos, a dye that localizes to the mitochondrion in response to its membrane potential. This colocalization assay shows that GatA is indeed a mitochondrial protein (Figure 4, A–C). Thus, both functional and localization studies and the nature of other mutants isolated by our screen establish that GatA has a mitochondrial function. Five alleles of *gatA* were isolated from our screen; we have located molecular lesions in four alleles (Figure 2). *JR94* has a 168-bp deletion in exon 5. *JR15* harbors a G > T transversion, resulting in the amino acid substitution Q54H. *JR113* has a C > G transversion that causes the substitution H278D. Finally, JV87 has three point mutations, G > T, T > G, and G > A, which result in the following amino acid changes: Q53H, A58S, and V74I. All of the mutations lie within the putative amidase domain of the protein.

ATL: It has been determined that *S. cerevisiae* has two genes encoding arginyl tRNA ligase (ATL), with one functioning in the cytoplasm and the other, *MSR1*, in the mitochondrion (Tzagoloff and Shtanko 1995). The Drosophila genome has three genes encoding proteins with potential arginyl tRNA ligase activity on the basis of Gene Ontology annotation in FlyBase (http://www.flybase.org): *atl*, *Aats-arg*, and *CG8097* (Drysdale and Crosby 2005). BLAST analysis shows that ATL is 36% identical to Msr1p, while AATS-arg and CG8097 are 28 and 24% identical, respectively. According to MitoProt analysis, ATL is only 8.4% likely to localize to the mitochondrion. This low number did not support a mitochondrial function for ATL. To pinpoint its subcellular location, we constructed an ATL–GFP fusion protein and demonstrated that it colocalizes with MitoTracker Orange CM-H2TMRos to the mitochondrion by immunostaining S2 cells transfected with the fusion protein (Figure 4, D–F). Thus, ATL's subcellular localization, similarity to Msr1p, and its suite of phenotypic similarities to other known mitochondrial mutants, indicate that this protein functions in the mitochondrion. Our screen has identified nine alleles of *atl*, and we have located molecular lesions in two of these nine alleles (Figure 2). *JR70* has a G > A transition resulting in the amino acid change G182R. *JV219* contains a 12-bp in-frame deletion starting at V266. Both mutations occur within the catalytic arginyl tRNA core domain.

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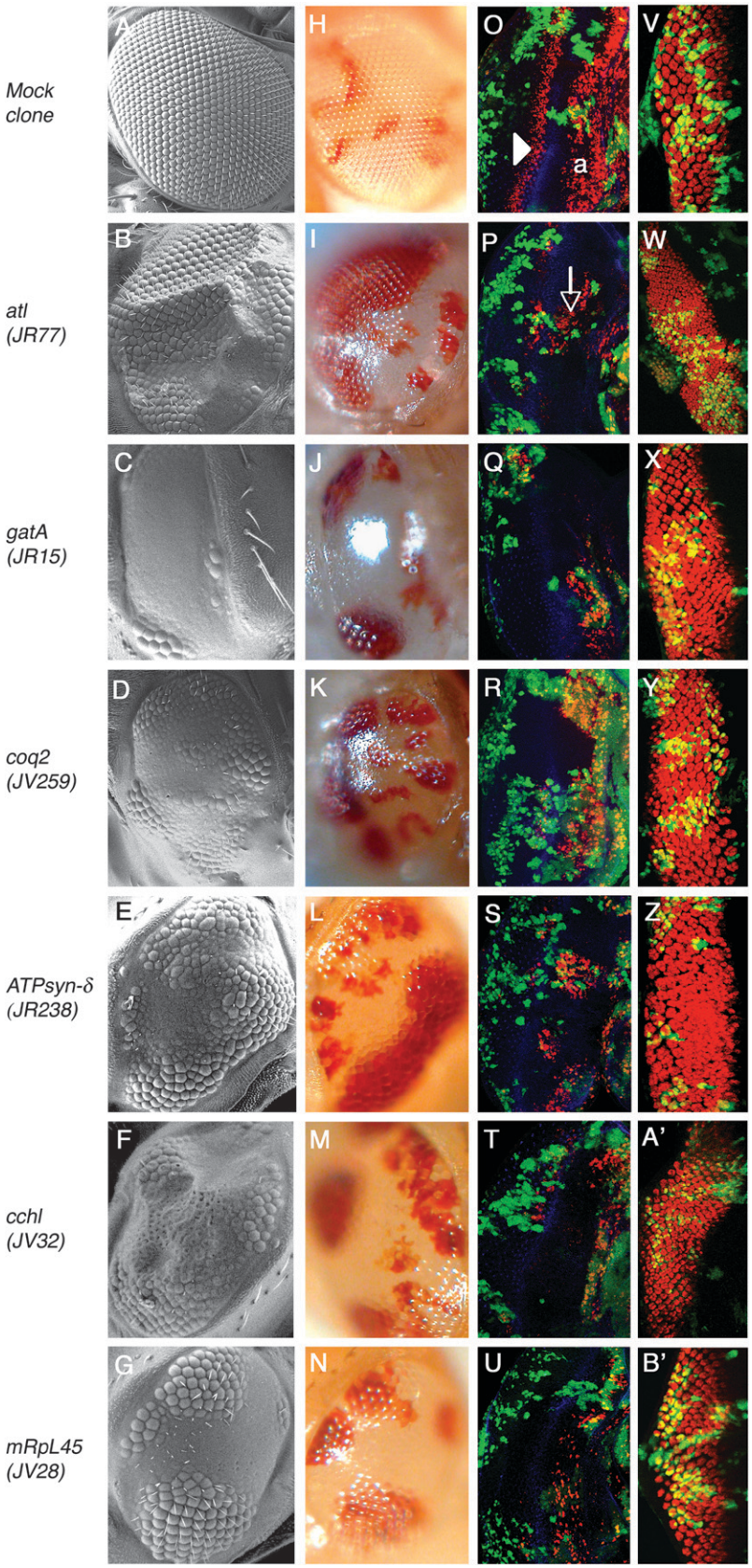

FIGURE 3.—Representative adult and larval phenotypes of mutants from mapped complementation groups. (A–N) Eyes of adult flies examined by scanning electron microscopy (A–G) and bright-field microscopy (H–N). An eye containing mock clones is mosaic in color (H), but the entire eye is faceted as in wild-type tissue (A). In eyes mutant for the indicated mitochondrial genes, cells heterozygous for the mutation are faceted (B–G) and red (I–N) as in mock clones, while homozygous clones are glossy and white. In all images, the representative allele is indicated in parentheses and posterior is to the left. (O–U) 30-min BrdU incorporation (red) in third instar larval eye discs with mock (O) and mutant (P–U) clones. Armadillo (blue) marks the morphogenetic furrow. In an eye disc with mock clones (O), both green and nongreen tissue are wild type. BrdU is randomly incorporated anterior to the furrow ("a"); posterior to the furrow, a single band of BrdU incorporation (arrowhead) marks the SMW. In mutant eye discs, BrdU incorporation is lost in homozygous mutant tissue, marked by the lack of GFP, both anterior and posterior to the furrow. Incorporation remains normal in clones heterozygous for the mutation (green). For comments on the apparent local nonautonomy of this phenotype (arrow), see MANDAL *et al.* (2005). (V–B′) ELAV antibody stainings of eye discs with mock (V) and mutant (W–B′) clones. Expression of ELAV is largely normal in all mutant clones.

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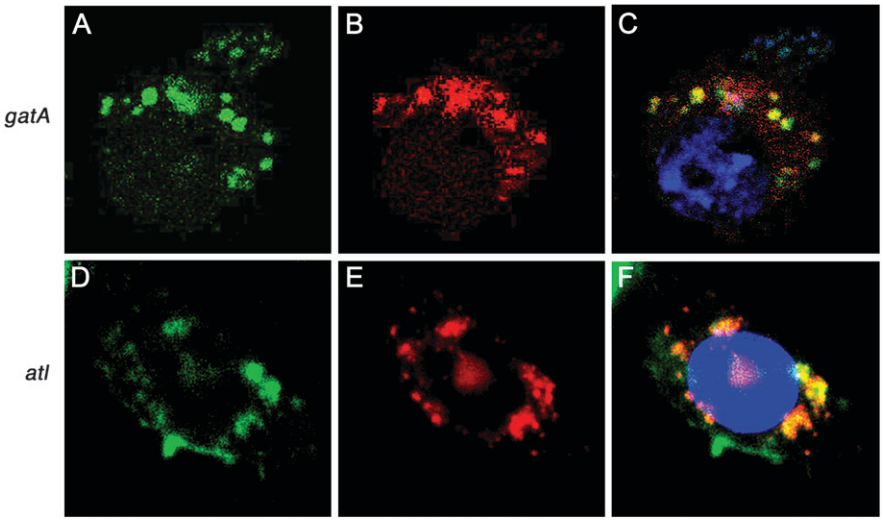

Figure 4.—GatA and ATL localize to the mitochondrion. Co-immunostaining of GatA–GFP and ATL–GFP fusion proteins (A and D, green), respectively, with MitoTracker Orange CM-H2TMRos (Molecular Probes, Eugene, OR) (B and E) shows mitochondrial localization of the fusion proteins (C and F, yellow). Full-length cDNAs were cloned using the Gateway cloning system (Invitrogen, San Diego) into the *pAWG* vector containing the *actin* promoter and sequence coding for a C-terminal GFP protein. Blue, DAPI staining (C) and TOPRO3 (F) staining.

mRpL45: mRpL45 is a protein of 599 amino acids with a molecular weight of 26 kDa (reviewed in Graack and Wittmann-Liebold 1998). At least 50 mitochondrial ribosomal proteins exist within the *S. cerevisiae* mitochondrial ribosome, among which at least 39 are classified as large subunit proteins. In humans, these ribosomal subunits help synthesize 13 mitochondrial proteins essential for oxidative phosphorylation, and mutations in the genes encoding them are associated with many disorders (reviewed in Sylvester *et al.* 2004). According to MitoProt, mRpL45 has an 88% chance of localizing to the mitochondrion. One mutant allele of mRpL45 was identified from the screen (Figure 2). *JV28* harbors a 9-bp deletion and a 7-bp insertion in exon 2 that results in a frameshift starting at V230 and a premature stop codon at the 279th amino acid. The mutation occurs within the TIM44 domain, which is involved in transporting the protein across the inner membrane of the mitochondrion (reviewed in Pfanner and Geissler 2001).

The generation of ATP in eukaryotic cells is largely dependent on oxidative phosphorylation in mitochondria (Figure 5). ATP originates from the oxidation of NADH and FADH2 via the electron transport chain, which consists of a series of four electron transporting complexes (I–IV) and the terminal complex V, which generates ATP. Complexes I and II transfer electrons, from NADH and FADH2, respectively, to ubiquinone (CoQ) via a series of iron–sulfur clusters. Ubiquinone, which is a mobile carrier, transfers these electrons to complex III. Cytochrome *c*, another mobile carrier protein, then delivers the electrons from complex III to complex IV where it reduces dioxygen to water. Concurrent with electron transport, complexes I, II, and IV pump protons from the matrix into the intermembrane space, forming an electrochemical proton gradient across the inner membrane, which is used to generate ATP by ATP synthase or complex V. Five mutants discussed in this study—*tend*, *pdsw*, *coq2*, *cchl*, and *ATP syn-δ*—function either within or between complexes of the electron transport chain. These mutants likely activate the G_1–S checkpoint as described for *tend* (Mandal *et al.* 2005). The remaining mutants—*atl*, *gatA*, *mRpL4*, *mRpL17*, and *mRpL45*—function in diverse roles within the mitochondrial protein biosynthesis pathways. The mitochondrial genome encodes 13 proteins that are essential for oxidative phosphorylation, and defects within the protein translation system are likely to cause downstream effects in energy production and subsequently affect the G_1–S transition of the cell.

The screen described here is the most efficient in any higher eukaryote for identifying nuclear-encoded genes that function in the mitochondrion. All six mutants mapped thus far are likely to have roles in mitochondrial processes. This is further supported by the fact that previously described *P*-element mutants in *pdsw*, *mRpL4*, and *mRpL17* also exhibit the same phenotypes as mutants isolated from our screen (Mandal *et al.* 2005). This is an independent confirmation that this screen is uniquely capable of selecting for disruptions in cell cycle regulation in the developing eye disc, caused by the activation of a metabolic checkpoint initiated by the mitochondrion. However, not all of the mutants isolated in this screen are expected to be disruptions in mitochondrial proteins. In Mandal *et al.* (2005), we described a pathway linking mitochondrial processes to cell cycle regulation in the nucleus. Two key members of this pathway, AMPK and p53, are known cytosolic proteins. This pathway is not yet fully understood, and we expect that our screen will also provide missing cytosolic links, in addition to mitochondrial ones described here. Our mapping results show that the screen is very likely to have identified some nonmitochondrial genes. For example, JV212 maps to 98E1-98E3 (Table 1, A and B), a region encompassing six genes, none of which bears homology to other mitochondrial genes or

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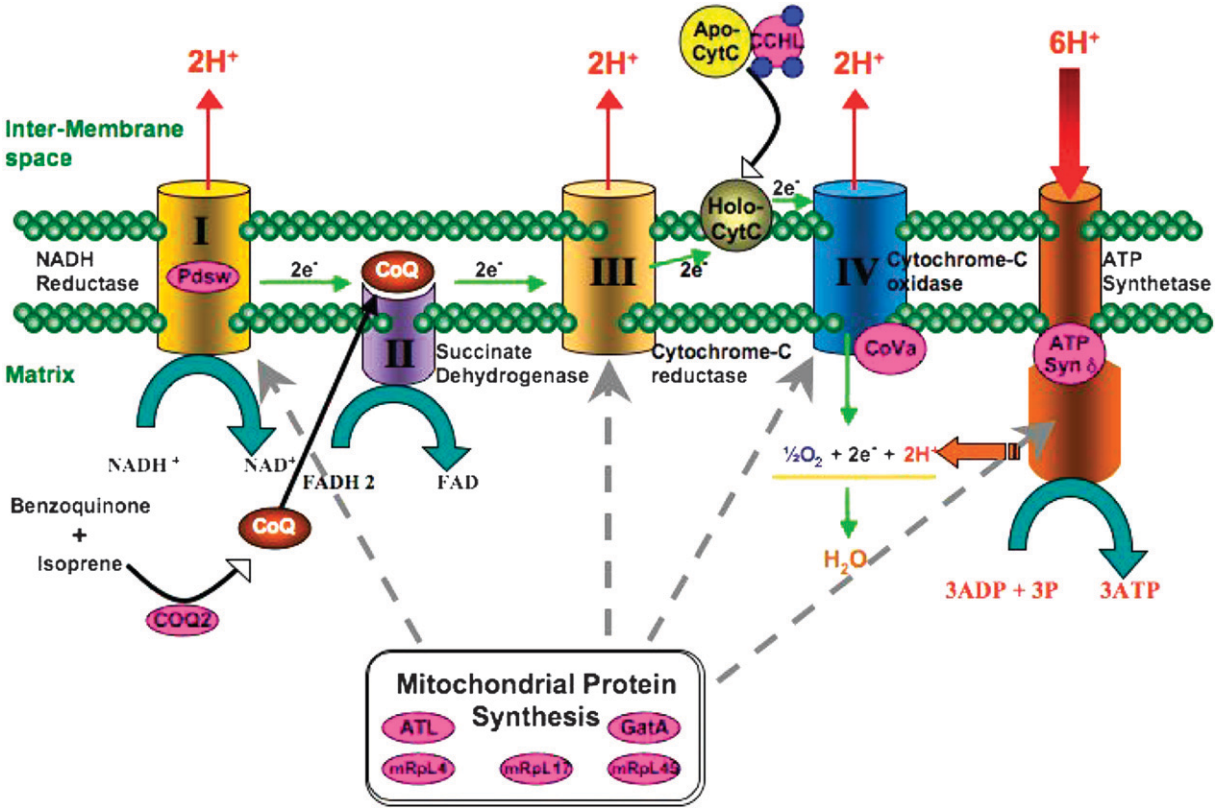

FIGURE 5.—Schematic of complexes involved in oxidative phosphorylation in the mitochondrion. Proteins highlighted in pink are encoded by mutant genes identified from this screen or described in MANDAL *et al.* (2005).

contains a mitochondrial localization sequence. This is again true for JR8 (Table 1, A and B), which maps to 100B9, another region containing five genes that are not likely to be mitochondrial.

An additional advantage to our screen is that it may be a more accurate predictor of mitochondrial function than the currently available *in silico* databases. For example, MitoProt predicts relative low probabilities of mitochondrial localization for CCHL (28%), Pdsw (10%), GatA (56%), and ATL (8.4%). We have demonstrated through immunostaining in this study that both GatA and ATL localize to the mitochondrion. Pdsw, a NADH dehydrogenase, and CCHL have been shown in previous functional studies to play roles in complexes I and IV, respectively, of the electron transport chain. Although MitoProt will be able to reliably predict mitochondrial localization for the proteins that fit its algorithm, our screen provides the more relevant *in vivo* information.

This study identified several new mutations in genes that function in either oxidative phosphorylation or mitochondrial protein biosynthesis. Consistent with our earlier observation using *tend* (MANDAL *et al.* 2005), these new mutants exhibit normal early cell divisions, a G_1–S block at the third larval instar, and proper neuronal differentiation. The new mutants help to further establish that attenuated mitochondrial function, caused by a loss in one of its many individual components, will result in an enforcement of the G_1–S checkpoint of the cell cycle. The fact that each of the characterized mutants with this suite of phenotypes encodes a protein with a mitochondrial function suggests that our method can serve as a general screen for identifying nuclear-encoded genes with mitochondrial function. This study represents yet another example of the power of Drosophila genetics and mutant screens in the discovery and refinement of important cellular processes.

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