

Spongecake and *eggroll*: two hereditary diseases in *Drosophila* resemble patterns of human brain degeneration

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Various neuronal degenerative diseases are characterized by late onset, relentless progression, and finally death. Many have a direct genetic basis; others are of still unknown etiological mechanisms [1,2]. The study of human neurodegenerative diseases is complicated by the difficulty of obtaining tissue samples at various stages of progression, especially early in the course of the disease. Since neurodegeneration occurs in many organisms [3–5], model organisms amenable to genetic and molecular techniques, such as the mouse, offer important advantages. Much less laborious and expensive are worms or flies, which have short generation times and can be rapidly screened for mutations. To investigate the use of the fly as a model system for identifying genes related to such diseases, we screened for mutants having reduced lifespan, then examined them for brain degeneration. We describe here two such mutants, each with a different pattern of degeneration as characterized by light and transmission electron microscopy. The brain of the aging *spongecake* mutant exhibits regionally specific, membrane-bound vacuoles similar to those seen in spongiform degenerations such as Creutzfeldt-Jakob disease [6,7]. The mutant *eggroll* develops dense, multilamellated structures in the brain, resembling ones found in lipid storage diseases such as Tay-Sachs [8].

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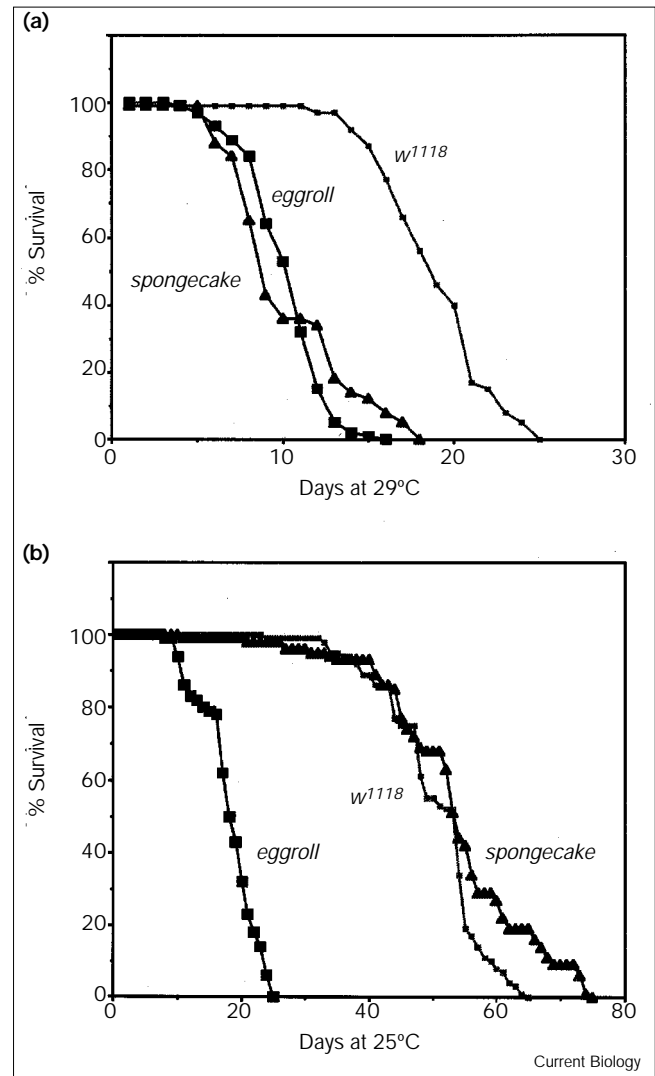
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Results and discussion

To obtain mutants, we set up some 5000 lines carrying ethyl methane sulfonate (EMS)-treated X chromosomes in a background of *w¹¹¹⁸*, a white-eyed strain of normal lifespan, and screened for mutations causing reduced adult lifetime at 29°C (after being raised at 25°C until eclosion). Approximately 100 flies of each strain were maintained at 29°C and scored for duration of survival. Candidates showing reduced lifetime compared with the parent strain were retested to confirm the phenotype, yielding 60 such mutant lines. These were examined after

Figure 1

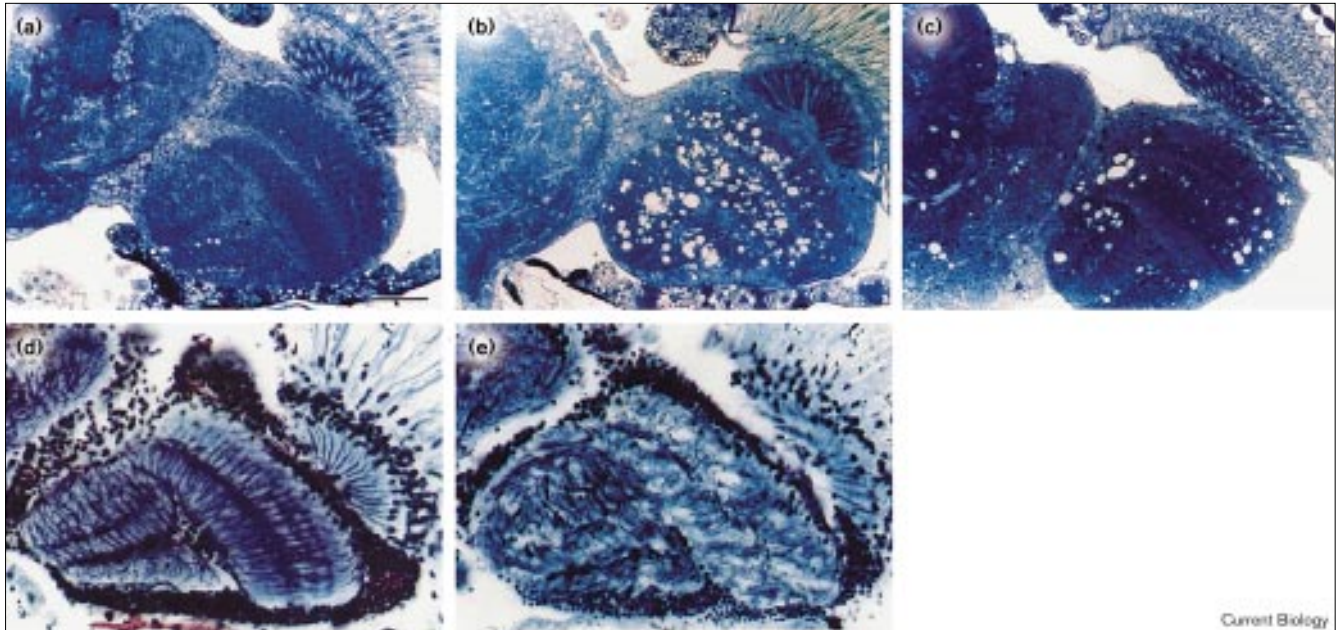


Reduced lifespan in brain degeneration mutants. Survival of the mutants *spongecake* and *eggroll* compared with flies of the parent strain (*w¹¹¹⁸*). Each initial population was approximately 100 flies, raised to adulthood at 25°C. (a) Newly eclosed adults placed at 29°C. (b) Maintained at 25°C. The phenotype of *spongecake* is temperature dependent.

aging, but before death, to identify those with brain degeneration. Two such mutants, both X-linked recessives, named *spongecake* and *eggroll* by virtue of their brain lesions, are described here.

Flies of the parent strain live for around 4 weeks at 29°C. In *spongecake* and *eggroll*, however, hemizygous male or

Figure 2



Brain degeneration in mutant flies aged at 29°C. (a–c) Horizontal sections (1 μ m) of plastic-embedded heads, stained with toluidine blue [3]. (a) Ten-day-old fly of the parent strain (*w¹¹¹⁸*). (b) Thirteen-day-old *spongecake* mutant; degeneration marked by vacuolization occurs predominantly in the synaptic neuropils of the optic lobes. (c) Ten-day-

old *eggroll* mutant. Vacuolization in this mutant extends over a wider territory. (d, e) Silver staining [15] of axons in the *spongecake* brain. (d) Newly enclosed mutant has normal axonal architecture. (e) After 13 days at 29°C, the axons have greatly deteriorated. Scale bar = 50 μ m.

homozygous female adults become sluggish in behavior a few days after eclosion and then begin to die; within 2 weeks most are gone (Figure 1a). Heterozygous flies live a normal life. The *spongecake* phenotype is temperature sensitive; at 25°C, the flies have a normal lifespan. When mutant embryos or larvae are reared at 29°C, there is also severe lethality, but not at 25°C. In *eggroll*, on the other hand, compared to the parent strain, lifespan is still greatly reduced at the lower temperature (Figure 1b).

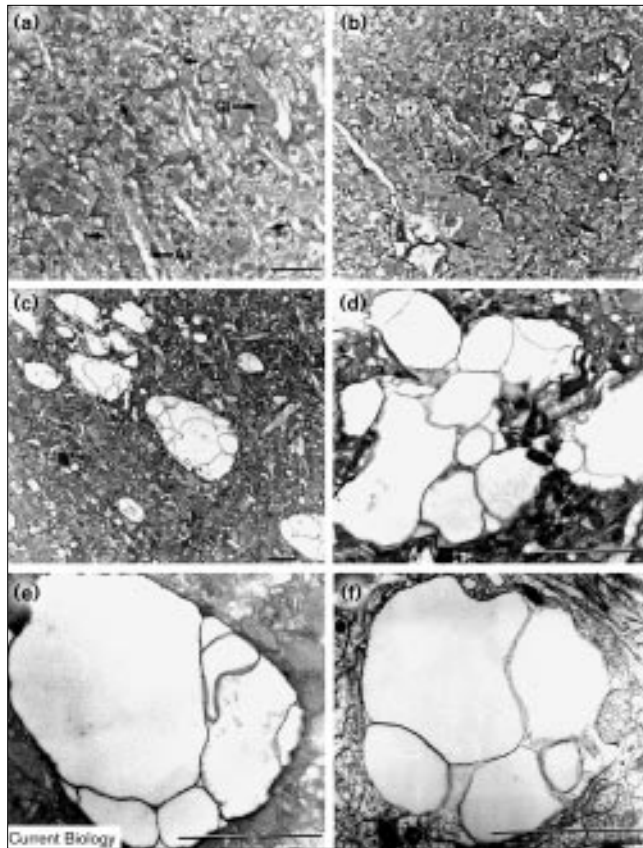
The recessive *spongecake* phenotype was rescued by the duplication *Dp(1;2)E1*, containing chromosomal bands 1A1–2A, whereas the deficiency *Df(1)sc[J4]*, in which bands 1B–3A3, are deleted, did not uncover the mutation, thus placing it between bands 1A1 and 1A10 on the X chromosome. The recessive *eggroll* mutation was uncovered by deficiency *Df(1)RA2*, which lacks 7D10–8A4, but not by *Df(1)KA14*, which lacks 7F1–8C6, thus placing the mutation between bands 7D10 and 7E11 on the X.

The brains of the two mutants and the reference line (*w¹¹¹⁸*) were examined at various ages by toluidine blue staining of semithin plastic sections for light microscopy, and by electron microscopy of ultrathin sections. The architecture of the fly brain is such that the neuronal and glial cell bodies are contained in cortical regions that surround synaptic neuropils (Figure 2a). The neuronal somata

and axons are enveloped by glial cell processes that can be identified by their greater electron density [9]. The neuropil regions contain intermingled processes of neurons, glia and synaptic connections [9,10]. In flies of the parent strain, few abnormalities were found in the various cortical and neuropil regions of either young or two week old brains. In *spongecake*, and *eggroll*, however, different patterns of age-dependent brain degeneration were found. Figures 2b and 2c illustrate advanced brain degeneration in aging *spongecake* and *eggroll*, as seen by light microscopy, compared with the normal fly shown in Figure 2a. There were no differences of phenotypes between males and females in both mutants.

spongecake mutant brains appear normal when young, but with age, vacuoles develop in the optic lobes, first in the medulla, then also in the lamina and lobula; the central brain is relatively spared. To test whether neuronal or glial cells are affected, we crossed the mutant with a line carrying *repo-lacZ*, which is expressed in glial cells. In the *spongecake* background, the *lacZ* staining pattern was essentially normal, indicating that the glial cells were unaffected. The neuronal somata were also normal. On the other hand, axons in the optic lobe showed severe damage, suggesting that the vacuoles come from axonal breakdown (Figure 2e). To characterize the early development of these defects at higher resolution, we used

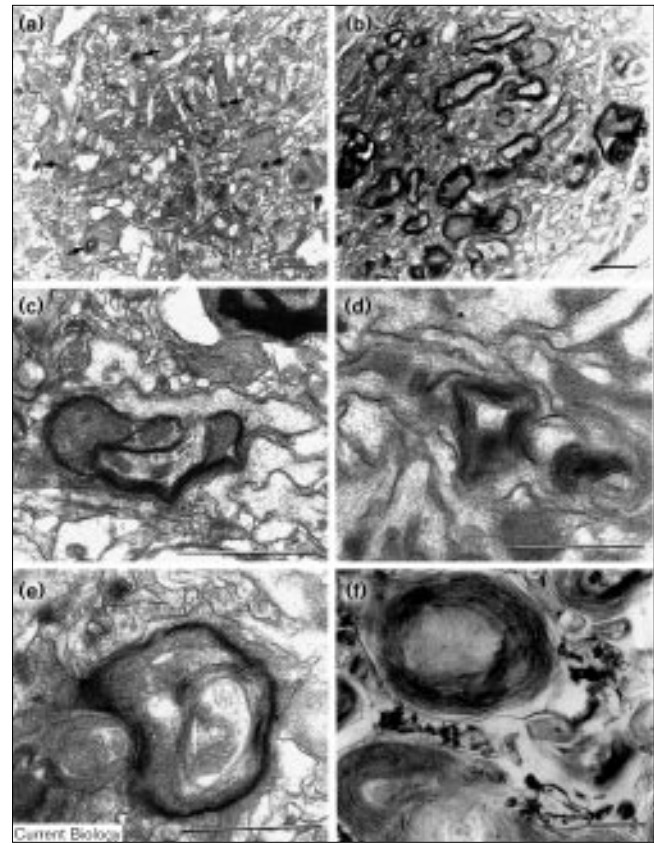
Figure 3



Ultrastructural defects in the *spongecake* mutant. (a) Medulla neuropil region of parent strain fly (after 13 days at 29°C), showing essentially normal structure consisting of intermingled axons (Ax), glial processes (Gl) and dark synaptic endings (arrows). (b) *spongecake* mutant after one day at 29°C. Two clusters of enlarged axons can be seen (arrows). (c) *spongecake* after 11 days at 29°C, showing increased number of abnormal structures. (d) Higher magnification of a cluster, apparently consisting of several enlarged axons, bounded by glial processes. (e) Coalesced vacuolar structure. (f) A similar membrane-bound vacuole in human brain neuropil associated with Creutzfeldt-Jakob disease, a prion disease (adapted from [7]). Scale bars = 2 μ m.

transmission electron microscopy. In *spongecake* adult flies, maintained at 29°C for one day from the time of eclosion, various axon terminals in the optic lobe neuropil become inflated. The swollen axons occur both singly and in clusters. These can be identified as axon terminals by their abundant synaptic vesicles and mitochondria at early stages. In some cases, groups of contiguous axons coalesce to form figures divided internally by membranes. These are presumably the precursors of the large vacuoles seen by light microscopy. The progression is shown in Figure 3b–e. The lesions in *spongecake* show a striking similarity to the membrane-bound vacuoles found in axonal terminals in Creutzfeldt-Jakob disease (Figure 3f), a human prion disease characterized by spongiform encephalopathy [6,7]. Although much investigation has centered on the

Figure 4



Ultrastructural abnormalities in the *eggroll* mutant. (a) Medulla neuropil in *eggroll* after one day at 29°C already contains cytoplasmic inclusion bodies (arrows); these occur in both neurons and glia. They appear to be precursors of the multilamellar structures seen in (b–e) in the neuropil after 9 days at 29°C. Intracellular structures often envelop mitochondria, as seen in (c) and (e, f). Concentric membranous cytoplasmic bodies (MCBs) in Tay-Sachs disease (adapted from [8]). Scale bars = 1 μ m.

causative agent, little is known of the underlying pathological mechanism responsible for the crippling inflation of the axons. While we cannot assume that the hereditary disease in the fly has the same cellular mechanism, mutants such as *spongecake* might provide a useful system for understanding basic processes that can cause such axonal inflation, as well as regional specificity.

In *eggroll* mutant flies, the phenotype is quite different from *spongecake*. Degeneration and premature death occur at both 25°C and 29°C. While one-day-old *eggroll* flies have essentially normal brain morphology at the light microscope level, by 4 or 5 days there is widespread degeneration, including the cortex, lamina, medulla, lobula, and central brain (Figure 2c). By 12 days, there is also retinal degeneration. Both male and female mutant flies show the same effects. Electron microscopy at early stages reveals that both neuronal and glial cells contain

cytoplasmic inclusions of a type that is rare in normal brain. Figure 4 shows these in the cortical regions of adult flies. As aging progresses, various types of multilamellar structures arise in the neuropil (Figure 4a,b). Some of these are within axons, surrounding mitochondria (Figure 4c,e). Inclusion bodies are also detectable in the brains of third instar larvae and pupae, even when raised at 25°C, indicating that development of the abnormality begins well before adulthood. Nevertheless, there is no lethality during development; survival rates at the various developmental stages show no difference from normal flies. As adults, however, these lesions are apparently associated with early death.

Dense, multilamellar inclusions resembling those in *eggroll* have been seen in many human disorders. For example, as illustrated in Figure 4f, concentric lamellated MCBs (membranous cytoplasmic bodies) are seen in the cytoplasm of cortical neurons in Tay-Sachs disease [8]. A mutant mouse with reduced lifespan that mimics human Niemann-Pick sphingomyelin storage disease also exhibits concentric multilamellar structures [11]. In aging monkey cerebral cortex, early changes include the formation of dense, multilamellar inclusions within presynaptic terminals and dendrites [12]. Most such structures in neuropathological diseases are lipid-related [13]. Mutants such as *eggroll* could be useful models for basic mechanisms in storage abnormalities. Further experiments will be aimed at identifying the biochemical natures of the various abnormal structures, and cloning of the *spongecake* and *eggroll* genes.

The two examples described here illustrate the effectiveness of our screening procedure, based on shortened lifespan, for uncovering genetic defects causing brain degeneration with advancing age. In the fly, the pathological mechanisms and progression of disease can be investigated from the earliest stages. The genes can readily be mapped and cloned, thus identifying a constellation of genes whose normal functions are needed to maintain the integrity of the aging fly brain. Past experience indicates that, in many cases, human homologues of fly genes are likely to be found [14], thus providing candidate genes for similar human syndromes.

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