Mitchell *et al.*, however, shows that genes that mediate differentiation can be trapped. The authors attribute their success to a potent drug-resistance marker that allows selection of genes with very low levels of transcription in ES cells.

Choose your mutagen

So how does gene trapping compare with chemical mutagenesis? The phenotype-driven screens rely on a phenotype, and the majority of the lines with no detectable phenotype obtained by gene trap would probably not be found after chemical mutagenesis. Thus, careful analysis of gene-trap lines might help the phenotypists to develop better screens. One disadvantage of gene trapping is that, in general, the only allele generated is a null allele. In contrast, chemical mutagenesis may produce partial loss-of-function mutations, and these can be useful in dissecting the different functions of a gene and its alternative splice variants. For example, an animal with a hypomorphic mutation may escape early lethality associated with a null allele, thus disclosing an affected process that occurs later in development.

On the other hand, chemical mutagenesis is stymied by the process of having to positionally clone the interesting mutations. The resolution of the mapping required, and how much needs to be done to reduce the candidate genes to a sufficiently small number for close examination to find the causative mutation, is a hot topic of much debate among aficionados. Both sides are signatories to the treaty establishing the International Mouse Mutagenesis Consortium[13], with the common goal of establishing a role for every mammalian gene. This challenge is of such a scale that every approach will be needed to tackle it. □

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A helicase is born

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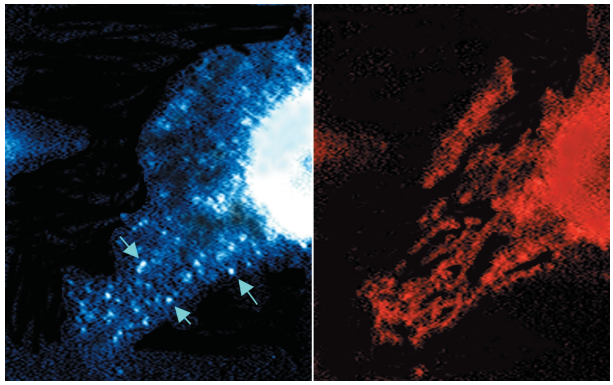
One of three loci previously associated with autosomal dominant progressive external ophthalmoplegia (adPEO) encodes ANT1, a mitochondrial nucleotide transporter. Now, mutations in two other genes are found in people with adPEO. One of these encodes a new helicase, Twinkle, which is related to the product of bacteriophage T7 gene 4, and co-localizes with mitochondrial DNA. The identification of Twinkle adds a new star to the expanding constellation of 'helicase diseases'.

For more than a decade, neuromuscular disease experts have been puzzled by a clinical syndrome characterized by the lack of eye movements—a condition known as progressive external ophthalmoplegia (PEO). In sporadic cases, each individual carries one deletion, which is found in the majority of her/his skeletal muscle mitochondrial genomes[1]. In most inherited cases, however, the muscle mitochondrial DNA (mtDNA) of each individual is damaged by many and diverse deletions[2]. This inherited disorder provided the first evidence that mutation of a nuclear gene can affect the integrity of mtDNA. On page 223 of this issue, Johannes Spelbrink and colleagues[3] provide a molecular clue about the nuclear-mitochondrial interactions underlying this disorder.



Mitochondrial star gazing. Twinkle is a novel helicase-like mitochondrial protein that co-localizes with the mitochondrial genome (immunostaining of bromodeoxyuridine-labeled cells; arrows in left panel). The mitochondrial marker in the right panel shows the organelle distribution. On page 223 of this issue, Spelbrink *et al*[3]. provide evidence that mutations in the gene coding for Twinkle are associated with multiple mitochondrial DNA deletions, and a neuromuscular disease, adPEO. Micrograph by Corina van Waveren (University of Miami).

Autosomal dominant inheritance of the disease is much more frequent than its recessive forms, and has been associated with three different loci in different families: 10q24, 3p14–21 and 4q34–35. The gene on chromosome 4q encodes adenine nucleotide translocator 1 (ANT1), a protein that controls ATP and ADP shuttling at the mitochondrial inner membrane in muscle cells[4]. The mechanism by which mutation of *ANT1* affects mtDNA integrity is unknown, but it may involve the stalling of the mitochondrial DNA polymerase γ, due to an imbalance in the mitochondrial deoxynucleotide pool.

Spelbrink and colleagues[3] have now identified the gene mutated in the 10q-linked adPEO families—*C10orf2*. This gene encodes a protein with homology to a T7 bacteriophage helicase/primase, the bifunctional enzyme encoded by the T7 gene 4. The homology, however, is significant

only in the helicase domain. Helicases are enzymes that can unwind duplex DNA or RNA molecules; DNA helicases mediate DNA replication, repair, recombination, and transcription[5], whereas RNA helicases are involved in transcription, RNA processing, regulation of RNA stability, ribosome assembly, and translation[6]. The *C10ORF2* gene product belongs to a class of hexameric helicases[7], and its amino acid sequence is well conserved among multicellular eukaryotes. DNA or RNA helicases exist in bovine and human mitochondria, but their function in mammals is poorly understood[8,9]. Two helicases have been characterized in the mitochondria of *Saccharomyces cerevisiae*, PIF1 and Hmi1p, neither of which has significant homology to the *C10orf2* product. Interestingly, Hmi1p is necessary for the maintenance of intact, but not partially deleted, mtDNA[10].

The *C10orf2* gene product co-localizes with mtDNA, giving rise to a punctate, star-like staining (hence Twinkle; see figure). Twinkle also co-localizes with mitochondrial nucleoids—ovoid bodies of about 0.3–0.6 μm in diameter that are well characterized in *S. cerevisiae*; where each nucleoid contains 3–4 copies of mtDNA and as many as 20 different polypeptides[11].

The association between *C10orf2* mutations and multiple mtDNA deletions is an enigma. As mtDNA deletions accumulate during aging of normal individuals, the effect of mutations in *C10orf2* may be associated with accelerated mitochondrial aging. This parallels the phenotypes of mutations in genes encoding some nuclear helicases of the RecQ group: Bloom syndrome[12] (characterized by immunodeficiency, impaired fertility, dwarfism and predisposition to cancer), Werner syndrome[13] (affected people age prematurely with graying and thinning of the hair, and are susceptible to developing cataracts, type 2 diabetes mellitus, osteoporosis, atherosclerosis and cancer) and some cases of Rothmund–Thomson syndrome[14] (which involves growth deficiency, photosensitivity of the skin, cataracts, early graying and loss of hair, and increased susceptibility to cancer). The precise function of these different nuclear helicases remains to be determined, but their mutant versions are linked, at the cellular level, by their common effects: increased genomic instability, including chromosomal breaks, multiple large deletions, and translocations[5]. If Twinkle proves to be a *bona fide* helicase, adPEO would be an additional helicase disorder associated with genomic instability and, as far as mitochondria are concerned, with premature aging.

On dominance and deletions

How could a dominant mutation in a helicase lead to increased mtDNA deletions? Spelbrink and colleagues[3] propose two potential mechanisms. Because Twinkle may be assembled as a hexamer (like its homologue, the T7 gene 4 product), mutations affecting subunit interactions could lead to a dominant-negative effect. They could not, however, detect a defect in the oligomerization of adPEO-modified Twinkle in cultured cells. Alternatively, mutations could lead to increased nucleotide hydrolysis, potentially altering the deoxynucleoside phosphate pool inside mitochondria. Indirect support for such a mechanism comes from the association with multiple mtDNA deletions of two genes involved in either the metabolism or the transport of nucleoside phosphates: *ANT1*, the adenine nucleotide transporter, and thymidine phosphorylase, a component of the thymidine salvage pathway, which is mutated in people with a disorder characterized by gastrointestinal symptoms[15].

It is therefore possible that an imbalance in the mitochondrial nucleotide pool is a common feature of diseases associated with multiple mtDNA deletions. Alternatively, multimeric Twinkle complexes containing defective subunits could be less processive when encountering different forms of DNA damage[16]. If Twinkle participates in the replication of mtDNA, these relatively rare events could lead to the stalling of polymerase γ and an increase in the recombination rate.

A link between defects in DNA polymerase γ activity and multiple mtDNA deletions is strengthened by the report of Gert Van Goethem and colleagues[17] on page 213. They have found that mutations in the DNA polymerase γ are associated with both adPEO and an autosomal recessive variant of the disease. Taken together, these reports confirm earlier suspicions that the mtDNA replication/repair machinery is involved in at least some cases of PEO with mtDNA deletions inherited as a Mendelian trait[2].

The next step will be to demonstrate that Twinkle is indeed a helicase *in vivo*. Spelbrink and colleagues[3] show that overexpression of a *C10orf2* cDNA in cultured human cells results in a very modest increase in helicase activity on artificial DNA/DNA templates *in vitro*. The possibility remains that Twinkle is an RNA helicase, or that it might use its helicase domain simply to bind DNA. Irrespective of its physiological function, the discovery of Twinkle and its mutant in adPEO is a significant step towards understanding the complex group of disorders involving nuclear-mitochondrial miscommunication. □

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