Deficiencies in sight with the candidate gene approach

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PHENOTYPES affecting the vertebrate eye are governed by a large number of genes, reflecting the complex biochemistry and cellular physiology necessary for vision. Mutations of these genes, many of which are expressed only in the eye, generally will not hinder the viability of individuals who carry them. The result, in humans, is a panoply of phenotypes ranging in severity from inconsequential (blue eyes or brown) to devastating (congenital blindness). This collection also includes genetic diseases that primarily result in the degeneration of the retina. Our understanding of them has been rudimentary and, until recently, biochemical defects were known for only a few rare forms of hereditary retinal degeneration in humans, such as Refsum's disease, abetalipoproteinaemia and gyrate atrophy. But now, they are yielding to the onslaught of molecular genetics technology. The most recent conquests are choroideraemia in humans, for which a candidate gene is reported by Cremers *et al.* on page 674 of this issue[1], and hereditary retinal degeneration in *rd* mutant mice, for which the precise biochemical defect is described by Bowes *et al.*[2] on page 677.

Gene isolation

Choroideraemia is a sex-linked form of hereditary retinal degeneration, the disease locus being on the long arm of the human X chromosome (Xq21). Males with the disease gradually lose vision in the mid-periphery of the retina, with a progressively decreasing island of central vision that is extinguished usually by the age of 60. The candidate gene was isolated using what has become the 'classic' approach to the cloning of a human disease gene based only on its chromosomal assignment. The first step is the laborious and sometimes serendipitous identification of DNA probes close (less than a few hundred kilobases) to the disease gene. A restriction map of the region surrounding the probe sequence is then constructed. Finally, one searches for messenger RNA transcripts derived from the region, usually by finding and using as a probe a sequence in the mapped region that has been conserved during evolution. Examples of human disease genes that have been cloned by variations of this approach are those for chronic granulomatous disease, Duchenne's muscular dystrophy, retinoblastoma, cystic fibrosis, DCC (for deleted in colon carcinoma), Wilms' tumour and von Recklinghausen's neurofibromatosis.

For the choroideraemia gene, the unknown sequence DSX165 was the key probe, as it can detect deletions or translocations of Xq21 in some patients with choroideraemia. About 45 kilobases of DNA surrounding DXS165 were mapped, and 15 single-copy probes isolated; two of the probes contained highly conserved sequences, and one of those two strongly hybridized with a distinct mRNA transcript expressed in the retina. Surprisingly, the gene is also expressed in cervical carcinoma (HeLa) cells and lymphoblasts. As Cremers *et al.*[1] point out, choroideraemia might therefore be a retinal degeneration secondary to a widespread metabolic defect, as is the case for Refsum's disease, abetalipoproteinaemia and gyrate atrophy. In fact, gyrate atrophy, which is caused by a deficiency of the enzyme ornithine aminotransferase, is clinically similar to choroideraemia, and in the past some ophthalmologists felt that choroideraemia and gyrate atrophy were forms of the same disease. The truth will be revealed in the near future, when the 'choroideraemia protein' predicted by the mRNA sequence is identified.

Despite the successes of 'reverse genetics', I believe that the identification of other human genes causing retinal degeneration (and perhaps many other human diseases) will come to rely more heavily on an alternative method, the 'candidate-gene' approach. With this technique one picks a cloned gene that is known to have a role in the physiology of the diseased tissue and then searches for mutations of the gene in patients with the disease in question. Recent technological advances for detecting point mutations in a candidate gene, such as those based on single-strand conformation polymorphisms[3] and direct sequencing of DNA using the polymerase chain reaction, make it possible to screen hundreds of patients, showing dozens of phenotypes, in a few months once the sequence and intron–exon structure of the gene is known. If a mutant sequence can be successfully correlated with a particular phenotype, then the known biological function of the candidate gene provides insights into the biochemical defect causing the disease. This approach conveniently bypasses the need for viable but diseased human retinas, which are available only rarely.

The candidate-gene technique is appealing to those enamoured of the scientific method, because it allows the formulation and testing of hypotheses about the aetiology of a disease on the basis of its clinical features and the role in the retina of a particular candidate protein. There are numerous candidate genes available for studies of the hereditary retinal degenerations. For example, there are cloned genes encoding proteins involved in phototransduction (opsin, transducin, 48K protein, phosphodiesterase and so on), vitamin A metabolism (interphotoreceptor retinol binding protein, cellular retinaldehyde binding protein) and the structure of the disk membrane (peripherin). Many of these proteins consist of separately encoded subunits, such as the α, β and γ subunits of transducin. Furthermore, some of the subunits are specific for certain photoreceptors — for example, the transducin α subunit differs between rods and cones.

Retinal abnormalities

Do naturally occurring mutations of any of these genes lead to retinal abnormalities *in vivo*, as one might expect? The answer is yes, because (1) a missense mutation of the rod opsin gene occurs in some patients with autosomal dominant retinitis pigmentosa, a form of retinal degeneration[4]; (2) the gene that is mutant in the *rds* (for retinal degeneration slow) mouse normally encodes the rod disk membrane protein peripherin[5]; and (3) Bowes *et al.*[2] now show that the gene encoding the β subunit of rod phosphodiesterase is responsible for autosomal recessive retinal degeneration in the *rd* mouse. Actually, before any of these discoveries in vertebrates, it was found that some *Drosophila* genes causing photoreceptor degeneration normally encode proteins involved in phototransduction or the cytoskeleton of photoreceptors[6–8].

For human retinal diseases, the task at hand is to use existing techniques to link each of the growing number of cloned candidate genes with one or more retinal degenerations or nonpathogenic phenotypes. We should expect that in the next decade many if not all of the genes involved specifically in retinal physiology will be found to be mutant in some people with visual deficits. For the longer term, let us hope that the knowledge gained from the resulting inventory of biochemical defects will provide clues to therapies for the patients concerned. □

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1. Cremers, F.P.M., van de Pol, D.J.R., van Kerkhoff, L.P.M., Wieringen, B. & Ropers, H.-H. *Nature* **347**, 674–677 (1990).
2. Bowes, C., Li, T., Danciger, T. L., Baxter, L. C., Applebury, M. L. & Farber, D. B *Nature* **347**, 677–680 (1990).
3. Orita, M., Suzuki, Y., Sekiya, T. & Hayashi, K. *Genomics* **5**, 874–879 (1989).
4. Dryja, T.P. *et al. Nature* **343**, 364–366 (1990).
5. Connell, G., Boscom, R., McInnes, R. & Molday, R.S. *Invest. Ophthalmol. Vis. Sci.* **31** (suppl.), 309 (1990).
6. Washburn, T. & O'Tousa, J.E. *J. biol. Chem.* **264**, 15464–15466 (1989).
7. Shieh, B-H., Stamnes, M.A., Seavello, S., Harris, G.L. & Zuker, C.S. *Nature* **338**, 67–70 (1989).
8. Montell, C. & Rubin, G.M. *Cell* **52**, 757–772 (1988).