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COMMENTARY

NOVEL APPROACHES FOR CLONING HUMAN GENES: THE CHRONIC GRANULOMATOUS DISEASE (CGD)

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The gene for chronic granulomatous disease (CGD), an X-linked inherited disorder of the oxidative system of phagocytes, has been cloned without the knowledge of its encoded protein. Deletion mapping analysis of patients with CGD and Duchenne muscular dystrophy (DMD) (patients BB and NF) has led to identification of the gene at the chromosomal location Xp21 (Francke *et al.*, 1985; Baehner *et al.*, 1986). Several molecular techniques were combined to isolate the gene.

Xp21 specific DNA sequences were isolated with the 'pert' hybridization and cloning technique. This method allows for the isolation of specific DNA sequences which are present in one and absent in another cell type (Kunkel *et al.*, 1985). Cells from patient BB, with an interstitial deletion of Xp21 DNA sequences, were used as cells lacking DNA and cells from a patient with 4X chromosomes were used as a source of DNA which contained these sequences. Genomic clones spanning about 10 per cent of the deletion were isolated, on the assumption that these might contain the gene among other sequences from the Xp21 region. To identify the gene among these sequences, knowledge of its tissue specific expression was used to identify potential clones, using a technique called subtractive c-DNA screening (Davis, 1984). It was expected that the CGD gene would be expressed in phagocytes, therefore a radioactive c-DNA was prepared from phagocytes (HL60 induced with DMSO) and subtracted with m-RNA from B cells from patient NF who had an interstitial deletion of Xp21 (cells that do not contain the m-RNA of the CGD gene). This subtracted c-DNA probe is specific for m-RNA transcribed from the Xp21 region and for genes expressed in HL60 but not B cells. The probe was used to screen Xp21 genomic clones. Two overlapping clones hybridized and further analysis showed that they contained parts of the CGD gene.

c-DNA clones corresponding to the entire transcript were isolated and used to analyse DNA and RNA from CGD patients. The gene is transcribed as a 4.8 kb m-RNA which is found in phagocytes and monocytes and to a low level in B cells but is absent in HeLa cells, fibroblasts, and kidney cells. One patient had a small deletion in this gene (Southern blot) and a deletion in m-RNA (Northern blot), which led to the absence of the carboxy terminus of the protein predicted from c-DNA sequence; three out of four patients had no m-RNA. The tissue-specific expression, chromosomal map position, and defects in the expression of this gene in CGD patients, indicated that the isolated gene is in fact the CGD gene (Royer-Pokora *et al.*, 1986). A definitive proof will require a functional assay in which defective cells from patients can be reconstituted with the cloned gene to yield a normal oxidative response (normal NBT test, Baehner and Nathan, 1968; Newburger *et al.*, 1979).

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Antibodies raised against a fusion protein or synthetic peptides will help to isolate the normal protein and to understand its role in the oxidative burst of phagocytes. Understanding the biochemical basis of the disease will help to explore new ways for therapy in CGD patients. It may even be possible to treat bone marrow precursor or stem cells of patients with CGD by gene transfer (Williams and Orkin, 1986). Identification of RFLPs in the gene will make both prenatal diagnosis and detection of carriers with a defective gene possible.

The successful cloning of the CGD gene was possible because several prerequisites were fulfilled:

1. A patient with a homozygous deletion was identified and B cells from that patient were established (these cells are negative for DNA and m-RNA sequences).
2. The gene was mapped to Xp21 and genomic clones from this region were available.
3. Expression of the gene was tissue-specific and large amounts of cells expressing the gene were available (induced HL60 cells).

Similar situations may be found for other genes and combinations of molecular techniques should lead to the isolation of a variety of genes without prior knowledge of their encoded gene products. Through reverse genetics, i.e. isolation of genes and analysis of DNA sequences and encoded proteins and use of natural mutations in the human population, light will be shed on many biochemical defects. Knowledge of the function of a given gene will contribute to the development of new therapy for human genetic diseases.

REFERENCES

Baehner, R.L., Nathan D., (1968). Quantitative nitroblue tetrazolium test in chronic granulomatous disease. *N. Engl. J. Med.*, **278**, 971–973.

Baehner R.L., Kunkel L.M., Monaco A.P., Haines J.L., Conneally P.M., Palmer C., Heerema N., Orkin S.H., (1986). DNA linkage analysis of X chromosome-linked chronic granulomatous disease. *Proc. Natl. Acad. Sci. USA*, **83**, 3398–3401.

Davis M.M., Cohen, D.I., Nielsen E., Steinmetz M., Paul W.E., Hood L., (1984). Cell type-specific c-DNA probes and the murine I region: the localization and orientation of A^d. *Proc. Natl. Acad. Sci. USA*, **81**, 2194–2198.

Francke U., Ochs H.D., De Martinville B., Giacalone J., Lindgren, V., Distèche C., Pagon R.A., Hofker M., Van Ommen G-J.B., Pearson P.L., Wedgwood R., (1985). Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome. *Am. J. Hum. Genet.*, **37**, 250–267.

Kunkel L.M., Monaco A.P., Middlesworth W., Ochs H.D., Latt S.A., (1985). Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. *Proc. natl Acad. Sci. USA* **82**, 4778–4782.

Newburger P.E., Cohen H.J., Rothchild S.E., Hobbins J.C., Malawista S.E., Mahoney M.J., (1979). Prenatal diagnosis of chronic granulomatous disease. *N. Engl. J. Med.* **300**, 178–181.

Royer-Pokora B., Kunkel L.M., Monaco A.P., Goff S.C., Newburger P.E., Baehner R.L., Cole F.S., Curnutte J.T., Orkin S.H., (1986). Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location. *Nature*, **322**, 32–38.

Williams D.A., Orkin S.H., (1986). Somatic gene therapy: current status and future prospects. *J. Clin. Invest.*, **77**, 1053–1056.