DNA typing to largely replace current DVI modalities. Until that time, as Olaisen *et al.* have demonstrated, DNA typing can be used as one of the principal DVI modalities. In this regard, commmunity mass disaster response plans should be revised and devised to reflect this reality.

Jack Ballantyne was the investigator in charge of the DNA identification of victims of the TWA Flight 800 disaster, which occurred off the shores of Long Island, NY in July of last year.

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PEX genes on the rise

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To the families of children afflicted with each of a dozen inherited disorders causing severe and progressive neurological deficits, the idea that yeast model systems might provide insights regarding the molecular basis of such diseases may conjure up a vision of clutching at straws. Yet, research over the past decade has forged precisely such a connection. Four papers in this issue of *Nature Genetics*, describing the molecular basis of two diseases, rhizomelic chondrodysplasia punctata (RCDP) and Zellweger syndrome (ZS), represent the early harvest of these endeavours[1–4].

Since the 1960s, clinicians have developed a growing appreciation for the toll exacted by a set of recessive human disorders that all revolve around the peroxisome, a subcellular organelle found in virtually all eukaryotic cells. These diseases are classified into three groups[5,6]. The first one, Group A, comprising ZS, infantile Refsum disease, neonatal adrenoleukodystrophy and hyperpipecolic acidaemia, is characterized by severe neurological and hepatic dysfunction, craniofacial abnormalities, and hypotonia, leading inevitably to early death. Patients accumulate phytanic acid and very long chain fatty acids (VLCFA) in the circulation and are deficient in the synthesis of plasmalogens, a class of ether phospholipids constituting up to 20% of our phospholipids. Patients in Group B, exemplified by RCDP, portray severe growth defects, rhizomelia, cataracts, epiphyseal calcifications and ichthyosis. These individuals accumulate higher levels of phytanic acid, and have normal levels of VLCFA but lack plasmalogens. The Group C disorders are characterized by a plethora of milder symptoms depending on the gene affected. While Group C diseases can be explained by mutations in single genes that compromise the activity or localization of only single enzymes, the disorders in Groups A and B affect the localization of multiple peroxisomal matrix proteins. The medical prognosis for these affected individuals is bleak, with little chance of survival beyond the age of ten, particularly for the Group A disorders. Although patients with many of the disorders die, the ability to propagate their cells in the laboratory has provided insights into the cellular defects. Cell-fusion studies place Group A disorders into 9 complementation groups (CG1-CG4 and CG6-CG10), and most Group B cells belong to CG11 (ref. 7).

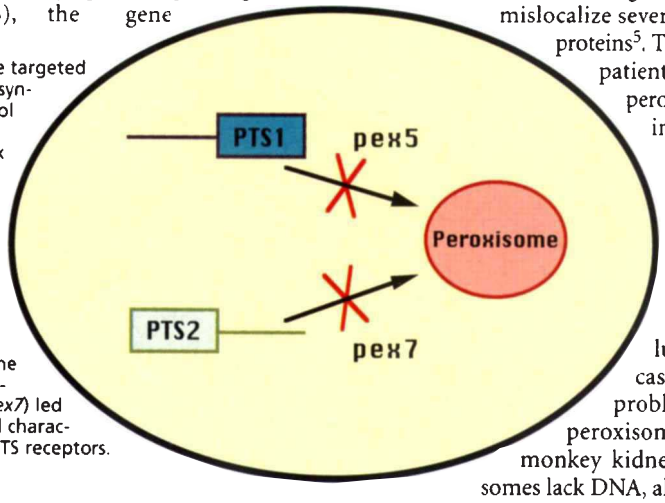

Fig. 1 Proteins are targeted from their site of synthesis in the cytosol to the peroxisome matrix *via* the use of either a C-terminal PTS1 or N-terminal PTS2 sequence. Yeast or human cells harbouring mutations affecting import *via* the PTS1-pathway alone (*pex5*) or the PTS2-pathway alone (*pex7*) led to the cloning and characterization of the PTS receptors.

Studies in the late 1980s showed that cells from patients with the Group A and B disorders contain peroxisomal membranes (called ghosts), but they appear to mislocalize several peroxisomal matrix proteins[5]. The hypothesis that these patients might be defective in peroxisomal matrix protein import could not be immediately tested because our knowledge about peroxisomal protein import at that time was meager.

A decade ago, a firefly enzyme, luciferase, unexpectedly cast light on the import problem. It was targeted to peroxisomes when expressed in monkey kidney cells. Since peroxisomes lack DNA, all of their proteins have to be encoded by the nuclear genome. These proteins were believed to be synthesized in the cytosol and imported post-translationally to the peroxisome. The peroxisomal targeting signal (PTS) in luciferase turned out to be a C-terminal tripeptide, SKL. This sequence, and its functional variants (collectively called PTS1), constitute the most prevalent PTS

Table 1 Conservation of peroxins and their role in human diseases

Homologous *PEX* genes	Function
PpPEX1 *ScPEX1*	117–127 kD AAA-family ATPase; cytosolic and vesicle-associated; involved in biogenesis
PpPEX2 *RnPEX2* *PaPEX2* ***HsPEX2***	35–52 kD C_3HC_4 zinc-binding IMP; human *PEX2* complements **CG10**
PpPEX3 *ScPEX3* ***HpPEX3***	51–52 kD IMP involved in biogenesis; mPTS in first 40 amino acids
PpPEX4 *ScPEX4*	21–24 kD peroxisome-associated ubiquitin-conjugating enzyme
PpPEX5 *ScPEX5* *HpPEX5* *YlPEX5* ***HsPEX5***	64–69 kD PTS1 receptor; 7 TPR domains; peroxisome-associated and cytosolic; human *PEX5* complements **CG2**
PpPEX6 *ScPEX6* *YlPEX6* *RnPEX6* ***HsPEX6***	112–127 kD AAA-family ATPase; cytosolic and vesicle-associated; involved in biogenesis; human *PEX6* complements **CG4**
ScPEX7 *KlPEX7* *MmPEX7* ***HsPEX7***	37–42 kD peroxisomal PTS2 receptor; 7 WD repeats; human *PEX7* complements **RCDP/CG11** lines[1–3]
PpPEX8 *HpPEX8*	71–81 kD peroxisome-associated protein; has a PTS1
PpPEX10 *HpPEX10*	34–48 kD C_3HC_4 zinc-binding IMP
ScPEX11 *CbPEX11*	27–32 kD peroxisome-associated protein involved in peroxisome proliferation
PpPEX12 *ScPEX12* ***HsPEX12***	40–48 kD C_3HC_4 zinc-binding IMP; human *PEX12* complements **CG3**
PpPEX13 *ScPEX13* ***HsPEX13***	40–43 kD SH3-domain-containing IMP; yeast Pex13p binds Pex5p
HpPEX14 *ScPEX14* ***HsPEX14***	39 kD peroxisomal membrane protein ; yeast Pex14p binds Pex5p and Pex7p[31,32]

Adapted from ref. 15. IMP, integral membrane protein; Pp, *Pichia pastoris*; Sc, *Saccharomyces cerevisiae*; Hp, *Hansenula polymorpha*; Cb, *Candida boidinii*; Yl, *Yarrowia lipolytica*; Kl, *Kluyveromyces lactis*; Pa, *Podospora anserina*; Mm, *Mus musculus*; Hs, *Homo sapiens*; Rn, *Rattus norwegicus*.

used for targeting to the peroxisomal matrix. A second sequence, PTS2, was uncovered in another peroxisomal matrix protein thiolase. This sequence, (R/K)(L/V/I)X_5(H/Q)(L/A), is found in a handful of peroxisomal proteins. Both PTSs are conserved from yeasts to humans[8]. Recently, mPTSs, responsible for targeting to the peroxisomal membrane, have been defined[9,10].

The use of heterologous peroxisomal matrix proteins such as luciferase and fusions of the PTS2 sequence to chloramphenicol acetyltransferase confirmed that cells from patients with Group A and B disorders were indeed defective in the import of PTS1 and/or PTS2-containing proteins[7,11–13]. The pleiotropic mislocalization of multiple proteins to the cytosol, and the normal targeting of membrane proteins to yield peroxisome 'ghosts', were rooted in the peroxisomal matrix protein import pathways.

The conservation of the PTSs led to the rational expectation that the mechanism of peroxisomal import was also conserved. Although the CG1-CG11 cell lines were available, the chromosomal locations of the genes responsible for these diseases were not, ruling out positional cloning, and leaving direct complementation as the only path to the affected genes. Several laboratories plunged into using yeasts such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha*, *Yarrowia lipolytica* and *Candida boidinii*[14] to study peroxisomal import and biogenesis. In the last 8 years, over 17 peroxins (proteins involved in peroxisomal import, biogenesis, proliferation and inheritance) have been identified, and the corresponding *PEX* genes cloned and characterized[15].

These yeast studies corroborated the existence of two distinct import pathways for matrix proteins, and pathway-specific mutants were defined (Fig. 1). The *pex5* mutant was defective only in the PTS1 import pathway, and not in the PTS2 or mPTS pathways[16,17]. Conversely, the *pex7* mutant was compromised only in the PTS2 pathway[18,19]. These mutants led quickly to the *PEX5* and *PEX7* genes, which encode the PTS1- and PTS2-receptors[20–22]. Several other mutants were affected in both import pathways, and are presumably crippled in common elements of the peroxisomal protein translocation machinery[14]. Like the human patient cell lines, the yeast mutants had peroxisomal membrane 'ghosts' and failed to import multiple matrix proteins. The premise on which the yeast work was founded had been substantiated, revealing the yeast mutants as excellent models for the cellular defect in human peroxisomal disorders.

Soon after the discovery of multiple import pathways in yeasts, it became evident upon further examination of the human CG1-11 cells that, like the yeast mutants, there were those affected in PTS1 import alone (CG2), or in PTS2 import alone (CG11), or in both import pathways (CG1, CG3, CG4, CG6–CG10)[7,13]. Most of the *PEX* genes were yielding homologues in several yeasts. That these genes were also conserved in humans came from the finding that the human homologue of yeast *PEX5* encoded the human PTS1 receptor[23–25] and that it was affected in CG2 (refs 23–24).

Three of the papers[1–3] in this issue prove that the human homologue of the yeast *PEX7* gene encodes the PTS2 receptor which, as predicted from its cellular import-deficiency phenotype, is the gene affected in RCDP, and the fourth paper[4] shows that the human homologue of the *P. pastoris PEX12* gene, encoding a zinc-finger-containing peroxisomal membrane protein, is affected in CG3. Of 17 *PEX* genes defined, most are conserved in yeasts and seven have human homologues (Table 1). Five of the seven human *PEX* genes are

implicated in peroxisome biogenesis disorders. It is a safe wager that these initial successes are just the tip of the iceberg. Additional homologues have appeared in mammalian EST databases, making the *in silico* entrée into the cloning of human peroxisomal disorder genes more efficient than the use of traditional complementation approaches, which have led to the definition of two *PEX* genes involved in disease[26,27]. Clutching at the proverbial straw has started a deluge!

While the evidence that human PEX7 is mutated in RCDP is convincing, the controversy surrounding the subcellular location and mechanism of action of this protein remains unresolved[8]. So far, the subcellular location of Pex7p in yeast and human cells has been determined only with epitope-tagged constructs[1,18,19], often under overexpression conditions[1,18]. It was concluded that the protein is predominantly cytosolic by one camp[1,18], and intraperoxisomal by another, which also described a new PTS located in the N-terminal 56 amino acids of ScPex7p[19,21]. Here the authors found that human PEX7, epitope-tagged at the N terminus, and overexpressed from the CMV promoter, is cytosolic[1]. Did the tagging at the N terminus, or the overexpression, cause the protein to appear cytosolic, or is this where the protein resides normally? Determining the location of native Pex7p in yeasts and humans is crucial in order to understand whether this receptor binds cargo in the cytosol and shuttles it to the peroxisome[18], or whether it behaves like an intraperoxisomal chaperone to pull cargo into the matrix[19].

Since proteins of the tetratricopeptide repeat (TPR) family often interact with WD40-repeat proteins, it seemed plausible that PEX5 (a TPR protein) might interact with PEX7 (a WD40 protein). Such an interaction was postulated in human cells to explain why a patient who did not express PEX5 was also deficient in the PTS2-import pathway[8]. The *S. cerevisiae* Pex5p and Pex7p do interact in a yeast two-hybrid system[2]. Such an interaction is unproven in mammalian cells, but the availability of the human *PEX7* gene should allow this idea to be tested.

Another puzzle is the fact that the *PEX5* gene is also essential for PTS2-protein import in humans[24], but not in yeasts[16,17]. A peroxisomal docking protein, Pex13p, for Pex5p was recently described[28-30], and *S. cerevisiae* has an additional peroxisomal docking protein, Pex14p, that binds both Pex5p and Pex7p[31]. In human cells, it will be interesting to see whether PEX14 binds both PEX5 and PEX7, or whether the proposed interaction between these receptors substitutes, or is essential, for the PEX7/PEX14 interaction. Yeast model systems will continue to be significant players in the elucidation of the biochemical functions of peroxins and their roles in human diseases.

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Human artificial chromosomes get real

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Recent advances in biotechnology have fueled significant progress in molecular genetics, ranging from the cloning of genes to the remarkable cloning of a lamb named Dolly from adult cells[1]. Nevertheless, there are many fundamental questions of human biology which remain unanswered. Of particular interest in human genetics are questions of chromosome structure and function. The basic DNA sequence requirements for human chromosome function are not precisely known, but are thought to be similar to those identified in yeast; namely a centromere, telomeres and origins of replication. More than a decade ago, these elements were characterized in yeast and subsequently used to construct yeast artificial chromosomes (YACs)[2–6]. YACs harbouring human genomic DNA are essential tools for human genome studies, including physical mapping and the identification, characterization and functional analyses of genes[7].

Following the success of YACs, many researchers have been trying to generate human artificial chromosomes (HACs), in the hope of defining the minimal sequence requirements for functional human chromosomal elements and providing improved understanding of chromosome behaviour. There is also the hope that functional HACs will serve as valuable non-viral gene transfer vectors[8]. An important step towards this goal is described on page 345 of this issue by Harrington *et al.*[9].

Of the three components thought to be necessary for human chromosome function (Fig. 1), only the telomeres are well characterized. Telomeres are DNA–protein complexes that serve to protect chromosome tips from end-to-end fusion and to prevent the loss of DNA during replication[8,10,11]. Telomeric DNA consists of long arrays (5–20 kb) of TTAGGG repeats. The ribonuclear protein polymerase, telomerase and the telomere repeat binding protein, TRF1, both appear to play a role in regulating telomere length[10–12].

In contrast to telomeres, human origins of DNA replication are not nearly as well understood. Origins are thought to occur