STUDIES OF RARE DISEASE LEAD TO DISCOVERY OF NEW COMPONENT OF HUMAN DNA REPAIR/ BASAL TRANSCRIPTION FACTOR.

Sometimes diseases are rare because they affect vital cell functions and only restricted alterations are compatible with survival. The papers in this issue by Jeffrey Ranish and colleagues[1] in Seattle and by an international team from the Netherlands, France and Italy led by Jan Hoeijmakers and Wim Vermeulen[2] describe the discovery of a new component of the DNA repair/ basal transcription factor TFIIH by studying yeast proteins and cells from patients with a form of trichothiodystrophy (TTD).

DNA REPAIR AND HUMAN DISEASE

This story begins in 1968 when James Cleaver of San Francisco published a seminal paper in Nature [3] describing a defect in DNA repair in cells from three patients with the rare disease xeroderma pigmentosum (XP) (Figure 1 top). This nucleotide excision repair (NER) system functions to remove photoproducts from DNA that is damaged by ultraviolet (UV) radiation. DNA repair is critical for all living organisms that are exposed to sunlight. XP patients who lack a normal functioning NER system are extremely sensitive to sun exposure, develop pigmentary abnormalities on their skin and have an early onset and a 1000-fold increase in sunlight induced cancers of the skin and eyes [4,5]. Subsequent investigations by scientists throughout the world revealed that the NER system in organisms as diverse as yeast, hamsters and humans is composed of a homologous series of proteins (Figure 1 bottom) [5,6]. These proteins function in concert to recognize DNA damage, unwind DNA in the damaged region, excise the damage creating an approximately 30 nucleotide gap, and filling in the gap using the undamaged strand as a template. Defects in seven of these proteins (XPA – XPG) are associated with the human disease XP.

The group led by Jean-Marc Egly in France was working on defining the basal transcription factor TFIIH when they reported in 1993 that two of the components were also DNA repair proteins: XPB and XPD.[7,8] This pivotal observation linked transcription to DNA repair since one protein functioned in both systems. This fact explained why mice completely lacking XPB or XPD were embryonic lethal since TFIIH is essential for survival. However, certain mutations in the XPB and XPD genes which are present in a few dozen patients with XP are compatible with survival[9]. Subsequently mice with these mutations were found also to survive and have some of the features of the patients[6]. However there are varied clinical phenotypes associated with different defects in the XPD and XPB genes in humans (Figure 1 top). Some involve progressive neurologic degeneration and others involve short stature, developmental delay, and cachexia (the XP/Cockayne syndrome complex). These appear to reflect defects in different functions of the same protein.

TRANSCRIPTION DEFECT IN TTD

One of the surprising observations made by Miria Stefanini and her group in Italy along with the scientists in the Netherlands and France was that some patients with defects in

XPD or XPB have the phenotype of trichothiodystrophy (TTD)[10-12]. These cells behaved in culture like XP cells but the patients have a very different phenotype with sulfur deficient brittle hair, skin photosensitivity without increased pigmentation and no increase in cancer susceptibility[5]. In 1993 they also identified a patient with TTD whose cells were hypersensitive to killing by UV but did not have defects in XPB or XPD[13]. This began a 10 year quest for the defective gene in TTD-A cells.

Since XPB and XPD were components of TFIIH, this complex became a candidate for the causative gene for TTD-A. Measurements of TFIIH showed low levels in TTD-A cells[14]. However, laborious sequencing of each of the 9 known components showed no mutations and addition of each of these purified TFIIH proteins did not correct the cellular defect [14]. Finally, new observations in yeast and in alga[15] came to the attention of the Dutch researchers. Ranish indicated that his group had found TFB5, a new component of TFIIH, in yeast. Like TTD-A cells, yeast cells that were deficient in TFB5 were hypersensitive to killing by UV. In addition, Rex1 a suppressor of UV sensitive mutants in the alga *Chlamydomonas reinhardtii*[15], had sequence homology to TFB5! They decided to exploit this information to determine whether the human homologue of TFB5 was defective in TTD-A cells. They assembled an international group of experts and used cellular, molecular and physical techniques to attain their goal. They cloned the new human factor (called TTDA), determined its size (8 kD), and its interactions with other human TFIIH components. They demonstrated functional correction of the DNA repair defect, the UV survival defect and the impaired TFIIH levels in TTD-A cells along with identifying mutations in this gene in cells from 4 affected patients in 3 families. Why was this important protein missed for so long? It seems that since it is so small – only 8 kD – that it was traveling with marker dyes in purification gels and was not visualized. However, persistence finally triumphed.

FROM BEDSIDE TO BENCH

These two papers illustrate the importance of studies of rare human diseases, of basic mechanisms of protein interaction in yeast, and of exchange of scientific information. Only 3 families are known with TTD-A but they provided information about functioning of transcription in all of humanity – indeed of most living eukaryotes. Similarly, the new technique of quantitative proteomics for the study of macromolecular complexes[1] has provided insights into important protein interactions. The varied clinical phenotypes represented in the top portion of Figure 1 may be the result of altered interactions of the defective proteins with others that control growth, development or cancer susceptibility. Only some of the proteins listed in the bottom of Figure 1 are currently associated with clinical diseases. The clinical phenotypes of the known disorders are very varied. Perhaps other rare diseases may result from defects in these proteins with phenotypes that we can only conjecture at this time.

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Figure 1. Relationship of some rare human diseases to defects in genes involved in DNA repair and transcription. Top: Seven human disorders (red rectangles) share the phenotype of sun sensitivity but differ in their involvement of the skin, nervous system, growth, development and cancer susceptibility. They are associated with defects in 11 genes (charcoal ovals). Bottom: The products of genes involved in the basal transcription factor TFIIH (yellow) interact with components of the NER system (other colors) to repair damaged DNA.