Title: Xeroderma Pigmentosum *GeneReview*: Functional Tests for XP
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Updated: August 2011

Note: The following information is provided by the authors and has not been reviewed by *GeneReviews* staff.

Functional Tests for XP

Functional tests on living cells, such as cellular ultraviolet (UV)hypersensitivity, unscheduled DNA synthesis (UDS), and host-cell reactivation, can be used to screen for abnormalities in DNA repair.[Kraemer & Ruenger 2008, Ruenger et al 2008, Stefanini & Kraemer 2008]. These assays were instrumental in research studies to unravel the genetics and pathogenesis of XP, but are not available clinically for diagnostic testing.

Note: In the XP variant, the clinical findings are the same as in other forms of XP; however, XP variant cells have normal nucleotide excision repair (NER) of UV-damaged DNA in contrast to cells from other forms of XP.

Cellular ultraviolet (UV) hypersensitivity. A post-UV exposure cellular survival curve reflects the ability of the DNA repair enzymes of a cell to repair UV-induced damage. Cellular UV hypersensitivity can be measured from skin fibroblasts [van Steeg & Kraemer 1999, Bootsma et al 2002, Kraemer 2003]:

- **XP cells** that show defective nucleotide excision repair (NER) are hypersensitive to killing by UV in comparison to normal individuals.
- **XP variant cells** may have normal or near-normal post-UV cell survival. However, post-UV cell killing of XP variant cells (but not of normal cells) is potentiated by addition of caffeine to the culture medium.

Unscheduled DNA synthesis (UDS). One of the most commonly used research tests of NER is UDS [Bootsma et al 2002, Kraemer 2003]. UDS measures the combined action of endonuclease, exonuclease, and polymerase in the NER system. Cells are treated with UV and then incubated in medium containing radioactive thymidine. The cells are then fixed and overlaid with x-ray-sensitive autoradiographic emulsion.

- **Normal cells.** UV radiation of normal fibroblasts results in a large increase in radiographic signal over all non-S phase nuclei.
- **XP cells.** UDS is abnormal. UV radiation of NER-defective (XP) fibroblasts results in minimal signal over non-S phase nuclei.
- **XP variant cells.** UDS is normal.

  Note: Alternative assays use scintillation counting to measure UV-induced incorporation of radioactive thymidine in non-dividing cells or immunofluorescence with non-radioactive ethynyl deoxyuridine (EdU)

[Limsirichaikul et al 2009] or BrdU [Hashimoto et al 2009] to replace the radioactive thymidine.

Host-cell reactivation. DNA viruses or plasmids do not have the ability to repair damage to their DNA and thus depend on host cellular repair systems [Emmert et al 2002]. Damaged DNA viruses or plasmids are expected to have greater growth or expression on cells with normal DNA repair capacity than on cells with reduced DNA repair capacity. A plasmid DNA repair assay utilizes a non-replicating plasmid that contains a reporter gene, such as luciferase. Host-cell reactivation involves transfection of a UV-damaged plasmid into the human host cell. The plasmid is repaired by the cell's enzymes. The activity of the reporter gene depends on the capacity of the DNA repair enzymes of the cell:

- **XP cells.** Host-cell reactivation is abnormal in all forms of NER-deficient XP. Expression of a UV-treated marker gene such as luciferase is lower in DNA repair-deficient XP cells than in repair-proficient normal cells.
- **XP complementation groups.** Host-cell reactivation is currently also used on a research basis to determine the XP complementation group by co-transfecting a UV-treated plasmid plus plasmids expressing wild-type XP cDNA of different complementation groups. [Emmert et al 2002].

Other Research Tests for XP

mRNA levels are reduced in many persons with mutation of *XPC* [Khan et al 2006] and *XPA* [Takahashi et al 2010] that result in a premature termination of transcription resulting in nonsense mediated decay of mRNA and subsequent lack of protein from the mutant allele. Such testing is available on a research basis only.

Western blotting indicates low level of XPC protein in persons with mutation of *XPC* [Khan et al 2006] or *XPA* [Takahashi et al 2010] or low levels of the protein DNA polymerase eta (Pol η; encoded by *POLH*) in persons with mutation of *POLH* that results in the XP variant phenotype [Inui et al 2008]. Reduced levels of XPC protein in fixed tissue from persons with mutation of *XPC* have been detected by immunohistochemistry [de Feraudy et al 2010]. Such testing is available on a research basis only.

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