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REPETITIVE DNA SEQUENCE IN IRRADIATED HYBRID CELLS

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Purpose:
Disruption of DNA primary nucleotide sequence is believed to be an important mechanism of radiation induced lethality. In vivo, DNA is maintained in a complicated structure that is essential to the integrity of the cell in interphase and during replication. The effect of irradiation on the conformation of the chromatin is unknown. This study was designed to detect and map alterations of chromatin produced by x-rays over regions of several million base-pairs.

Materials & Methods:
Human-rodent hybrid cells (HHW141) produced by exposure of human cells to high doses of x-rays, fusion with hamster cells (CHO), and temperature selection, contain a single human chromosome detectable by cytogenetic analysis. Megabase restriction mapping of these hybrid cells with the adenine-methylase M. *Taq* I plus the adenine dependent-endonuclease *Dpn* I was performed to extract large fragments of DNA that was unmethylated at the CpG dinucleotide. Rearrangement of the DNA produced by x-rays on this megabase region was detected by pulsed-field electrophoresis, Southern blotting ("zoo blots"), fluorescent in-situ hybridization, cloning and sequencing.

Results:
A 600kb structure, maintained unmethylated at the CpG dinucleotide in the hybrid cells, was isolated and defined. The region was found to be made up of repetitive units of approximately 2000bp. The 2000bp region had a basic unit of 300bp. The 300bp region contained rearranged DNA that included telomere-like sequences and sequences homologous to mitochondrial DNA. In situ hybridization gave a unique and reproducible pattern that covered diffuse areas of chromosomes in human and several other mammalian cell lines.

Conclusion:
The enzymology employed in these experiments can detect genetic alterations that can not be seen by traditional methods of genetic analysis. Irradiation can result in the rearrangement of large regions of DNA between cellular compartments and these new structures can be maintained in the cell. It is possible that these rearrangements and repetitive regions allow enhanced expression of an element that offers the hybrid cells an advantage in the subsequent selection. This system can be used as a model for x-ray induced alterations in chromatin structure.

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Title: MOLECULAR MECHANISMS FOR TRANSCRIPTIONAL CONTROL OF STRESS GENES.

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There are a number of families of stress genes that have been identified in eukaryotic cells. The two most prominent members are those encoding the heat shock proteins (hsps) and those encoding the glucose regulated proteins (grps). Expression of the hsps and grps occurs after the cells have been exposed to a particular type of stress. For example, oxygen deprivation induces grp synthesis whilst re-oxygenation causes hsp synthesis. Transcriptional regulation of these proteins is under the control of protein factors (transcription factors) which bind to the promoter region of these genes and stimulate mRNA synthesis. We have investigated the transcriptional regulation of both the grps and hsps under stress conditions.

(i) Expression of the grps, which encode major structural proteins in the ER, is greatly increased after cells are exposed to agents such as A23187 or tunicamycin, which inhibit ER function. Accumulation of GRP78 mRNA requires chronic (<2 hours) exposure to these compounds and the increased expression of GRP78 and GRP94 after addition of these compounds is blocked by pre-incubation in cycloheximide. Activation of grp transcription requires the modification of a pre-existing transcription factor which is already bound to the grp gene. This factor is monomeric (110kd protein) and may require phosphorylation to activate transcription.

(ii) Expression of the hsps is greatly enhanced after heat shock, and these proteins may serve to protect the cell from thermal damage. Binding of the heat shock transcription factor (HSF) to the promoter of the heat shock genes occurs only after exposure to heat. The active HSF is formed by the heat- and Ca^{2+}- induced oligomerization of the monomeric HSF (92kd) into an active hexamer (approx. 600kd). A further phosphorylation may be required, since activation of the hsp can then direct transcription of the hsps.

We conclude that there are divergent pathways for activation of the stress genes, indicating that the cell is able to sense different types of stress and activate the appropriate mechanism to deal with it.