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PRINCIPAL INVESTIGATOR: Robert W. Harrison, III, M.D.

CONTRACTING ORGANIZATION: University of Rochester
Rochester, New York 14627

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Rochester Rochester, New York 14627 E-MAIL: robert_harrison@urmc.rochester.edu		
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13. ABSTRACT (Maximum 200 Words)
 The purpose of this project is to identify the androgen-regulated genes in LnCaP cells that might play a role in the regulation of prostate cancer growth. The genes were identified by gene trapping in which LnCaP cells were transfected with a selectable gene driven by a defective promoter so that only cells in which the transgene could utilize endogenous promoter elements were selected. The specific gene could then be identified by gene walking from the transgene into the surrounding endogenous DNA. At least fifty LnCaP cell clones have been identified in which androgen down-regulated promoter elements have been trapped. Two genes have been identified by gene walking thus far. One is the gene for a casein kinase previously described as expressed in the prostate and androgen regulated. The other is the gene for a peptide elongation factor EIF4A2. EIF4A2 is described as more highly expressed in skeletal muscle and prostate but has not been previously reported as androgen-regulated. These results suggest that gene trapping is a viable method of identifying hormone regulated genes and that there remain androgen-regulated genes to be discovered.

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Progress (Headings are from Statement of Work):

Produce ~90 LnCaP cell clones containing trapped up-regulated genes and ~ 90 LnCaP cell clones containing trapped down-regulated genes.

This objective has been accomplished. LnCaP cells have been successfully transfected with the pHyTKpcs transgene and over 50 cell clones isolated.

Using "Easy Gene Walking" and lysate from each clone, sequence at least 200 bp of the DNA 5' of the trap transgene to eliminate any duplicate clones.

DNA adjacent to the inserted transgene was sequenced by gene walking (Harrison, et. al., 1997). The results are summarized below. More negatively-regulated colonies were recovered than positively regulated ones. Of the negatively-regulated clones, PCR of 60 DNA samples produced 30 bands that could be isolated and sequenced. In 15 cases, the adjacent DNA contained plasmid sequences suggesting that the vector had recircularized before insertion into the genome. Another eight bands provided three unique sequences.

LnCaP Gene Trapping Results

	Negatively Regulated	Positively Regulated
Total Colonies	60	25
Sequenceable DNA Bands	30	7
Plasmid Sequences	15	1
Identifiable Sequence	4	1

None of the three unique sequences appeared fully homologous to a human Genbank entry although some matches were found. In instance A, a sequence of 531 nucleotides contained a 411bp stretch that had an 86% homology with the mouse gene for protein synthesis initiation factor 4A. In instance B, 194 of 343bp had a 96% homology with a rat casein kinase and a 100% match to 44 bases of a patented human sequence. Lastly, instance C had only a 75.8% identity in a 120 nt overlap.

FASTA Search Results for Three LnCaP Genes Found by Trapping

A	GB_RO:MMEIF4AII , <i>M. musculus</i> eIF-4AII gene for protein synthesis initiation factor, 85.6% identity in 411 nt overlap GB_PR2:S79942 , EIF4A2=human protein synthesis initiation factor 4A-II homolog, 98.9% identity in 88 nt overlap
B	GB_RO:RATCKID , Rat casein kinase I delta mRNA, complete cds, 95.4% identity in 194 nt overlap GB_PAT:I92777 , Sequence 43 from patent US 5728806, 100.0% identity in 44 nt overlap
C	GB_PR3:HUAC002990 , Human Chromosome 16 BAC clone CIT987SK-A-1000D7, complete sequence, 75.8% identity in 120 nt overlap

A. The ARelong Gene

The PCR fragment produced by gene walking using DNA from the LnCaP/N12 clone was over 400bp in length. Nearly the entire length demonstrated significant homology to a highly conserved mouse gene (eIF-4AII) for protein synthesis initiation factor 4A and there was a 98.9% identity in a 88 nt overlap for the human gene, GB_PR2:S79942. This suggests that LnCaP cells may contain an androgen-regulated initiation factor that is similar, but not identical, to protein synthesis initiation factor 4A-II. Protein initiation factors have been previously shown to be involved in androgen-regulated prostate growth and many of the genes from this family encode a DEAD box protein/RNA helicase and are involved in cell cycle regulation (Kim et al, 1993; Nielsen and Trachsel, 1988; Sudo et al, 1995).

B. The ARkinase Gene

The sequence matches obtained using the FASTA search are shown below. The PCR fragment produced by walking using DNA from the LnCaP/N12 cell clone was ~343 bases long and consisted of 79 bases of the POMC promoter, followed by ~70 bases of plasmid sequence and ending in ~200 bases that have extensive homology with the Rat casein kinase I delta clone designated GB_RO:RATCKID. The sequence lacking the POMC and plasmid sequence is shown below, matched to the rat casein kinase. Only 10 bases differ out of 193. Since there was not homology with any components of the trap transgene, we conclude that this gene, that we have termed *Arkinase* is a newly-discovered, androgen-regulated kinase expressed in the prostate epithelial cell.

Are any clones observed to grow more slowly than others? If so, perform growth curves using charcoal stripped serum containing $0-10^{-7}$ M dihydrotestosterone and compare growth of the trapped gene clone with parental LnCaP cells. Otherwise, perform steps 4 & 5. For clones exhibiting defective growth, perform steps 4 followed by 9-12.

Slow growing clones were observed but could not be harvested in sufficient amounts to allow sequencing by our standard method. If time permits, this objective may be revisited.

Obtain a total of 1000-2000 bp of sequence of each trapped gene. Use this information to search data banks for matches and, with PCR, to produce Northern blot probes based on the gene walk sequence.

Two of three clones match open reading frames of genes archived in GenBank indicating that the sequences obtained by us contain open reading frames as well. Therefore, the ARkinase and ARelong sequences will be used to probe LnCaP mRNA. Sequence "C" will be extended.

Statement of Work Items Remaining to be Done

Perform Northern Blots using RNA from the androgen-responsive LnCaP cells and RNA from the androgen-insensitive cells PC-3 and DU 145. Probe the blots using the gene fragments created by PCR.

For probes that do not identify an RNA species, consider additional sequencing or sequencing from the 3' end of the trap transgene to obtain coding sequence.

For probes that identify an RNA band that is not dihydrotestosterone regulated: discontinue evaluation of that cell clone.

For probes that identify an RNA band that is dihydrotestosterone regulated in insensitive as well as sensitive cells: retain for possible future evaluation in other androgen-responsive tissues.

For probes that identify an RNA band that is differently regulated in LnCaP cells vs the insensitive cells: Use Northern blots of human tissues to determine the tissue distribution of the trapped gene and use the probe to identify and isolate clones from a prostate cDNA library giving highest priority to regulated genes expressed in few tissues.

Using one or more isolated cDNA clones from #9, obtain a full-length sequence and construct a full-length clone.

Use the protein sequence derived from the cDNA to design one or more peptides to produce antisera for use in Western blotting experiments to confirm that the gene product is regulated similarly to its mRNA.

Insert the cDNA into a expression vector driven by the CMV promoter or synthesize antisense oligomers to the candidate gene. Use androgen-independent expression of the gene or reduction of the gene's expression to test the effect on growth.

KEY RESEARCH ACCOMPLISHMENTS:

- Gene Trapping of androgen-regulated genes in LnCaP cells was accomplished
- At least two genes, not previously identified as expressed or regulated in LnCaP cells have been identified.

CONCLUSIONS:

- Gene trapping is a viable method of identifying hormone regulated genes
- Androgen-regulated genes exist in LnCaP cells that have not been previously described.
- The gene trapping procedure may preferentially identify exonic sequences.

REPORTABLE OUTCOMES:

Abstract only-data too preliminary.

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APPENDICES:

Harrison RW Eyo U Gillespie K (2000) **Identification of Androgen-Regulated Genes in LnCaP Human Prostate Cancer Cells by Gene Trapping**. *Proceedings of the 82nd Annual Meeting of the Endocrine Society*, pp359.

Identification of Androgen-regulated genes in LnCaP Human Prostate Cancer Cells by Gene Trapping. Robert W. Harrison, III and Unwana Eyo and Kathleen Gillespie. University of Rochester School of Medicine and Dentistry. Rochester NY 14642.

Although treatment of prostate cancer by androgen withdrawal has been an established treatment for decades, the molecular basis for androgen-dependent prostate growth is unclear. Since androgen effects are mediated through changes in gene expression, the molecular basis for prostate cancer regression upon androgen withdrawal and eventual escape from withdrawal would be better understood if the genes regulated by androgen in the prostate were known. This laboratory has developed a method of identifying hormone-regulated genes by "trapping" (Harrison & Miller. *Endocrinology*. 137:2758, 1996). The "trap" is a selectable transgene which lacks a functional promoter. After stable transfection, a two-stage selection is done in which only cells containing a transgene that has usurped the functions of a native, regulated promoter are left alive. The flanking, native DNA is then sequenced by gene-walking (Harrison et al, *Biotechniques*. 22:650-3, 1997). Over 100 LNCAP clones have been isolated of which ~80 contain trapped, down-regulated genes and ~30 contain trapped, up-regulated genes. Initial walks have been performed on a total 20 DNA samples of which one is homologous to a gene not previously described as androgen-regulated. This gene has homology with known protein synthesis initiation factors and thus, may play a supporting role in androgen stimulation of prostate protein synthesis.