DE91 005006-2

MONOCHROMOSOMAL HYBRIDS FOR THE ANALYSIS OF THE HUMAN GENOME

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DE91 005006

PROJECT SUMMARY

In this research project we have proposed to develope rodent/human hybrid cell lines each containing a single different human chromosome. The human chromosomes will be marked with Ecogpt and stably maintained by selection in the hybrid cells.

The experimental approach to produce the proposed cell lines involve the following: We will first transfer a cloned selectable marker, Ecogpt (an E. coli gene for xanthine-guanine phosphoribosyltransferase: XGPRT) to normal diploid human cells using a retroviral vector. The transferred gene will integrate at random into multiple sites in the recipient cell genome. Clonal cell lines from independent transgenotes will each carry the selectable marker integrated into a different site and perhaps a different chromosome. The chromosome carrying the selectable marker will then be transferred further to mouse cells by microcell fusion. In addition we will also use directed integration of Ecogpt into the chromosome present in rodent cells, otherwise not marked with a selectable marker. This will allow us to complete the bank of proposed cell line.

The human chromosome, since will be marked with a selectable marker can be transferred to any other cell line of interest for complementation analysis. Clones of each cell line, containing varying size segments of the same chromosome produced by selection for the retention or loss of the selectable marker following X-irradiation or by metaphase chromosome transfer method will facilitate physical mapping and determination of gene order on a chromosome.

TECHNICAL REPORT

Objective: The objective of the research project is to transfer individual human chromosomes to mouse and/or Chinese hamster cells to produce hybrid cell lines each harboring a single different human chromosome. The human chromosome present in rodent cells will be marked with a dominant selectable marker and maintained by selection. These cell lines will serve as a national resource for mapping and sequencing the human genome.

Methodology: The details of methodology has previously been described in the original grant application. In summary, we will first transfer a dominant selectable marker into a human lymphoblastoid (L1265, L1343 and L8282) and a normal fibroblast cell line using retroviral vectors. The retroviral vectors available in our laboratory are modified to clone genes for xanthine guanine phosphoribosyl transferase (Ecogpt) and resistance to antibiotic hygromycine (hygro) together. These vectors are transfected into a packaging cell line (PA 317) to produce infective virions. The vectors packaged as virus particles are infected into human cells to produce a bank of cell lines each containing a single different marked chromosome. The marked human chromosomes are then transferred to rodent cells by the method of microcell fusion. An outline of the methodology is given in figure 1.

Progress Report: **The progress made towards the final goal since the inception of the project is as follows:**

1. We have constructed the necessary vector system for integrating the selectable marker into the human chromosomes.

2. We have recovered at least 102 independent clones of a fibroblast cell line each carrying the marker integrated into a different site. These clones are being analyzed to identify 24 cell lines each carrying the marker integrated into different chromosome.

3. A mixed population of lymphoblastoid cells carrying the selectable marker has also been produced. These cells will be fused with a mouse cell line to rescue the marked chromosome into mouse/human hybrid cells.

4. We have developed an experimental strategy using PCR technology to rescue the human DNA sequences that flank the integrated selectable marker. DNA sequences recovered from independent clones, each carrying the marker at a different site, are used as probes to identify the chromosome of origin. This will allow us to identify 24 clonal cell lines each carrying the selectable marker into a different chromosome. Once identified, each chromosome will be transferred by microcell fusion into mouse or hamster cells. We have already applied this system to identify marked chromosomes in six clones.

5. Using marked lymphoblastoid cells and clones of fibroblasts we have produced a total of 12 monochromosomal hybrids to date. These hybrids include:

Hybrid	Description	Human Chromosome
RA2-1	Mouse/Human	2
RA 7	Mouse/Human	7
RA 9	Mouse/Human	9
RA 15	Mouse/Human	15
RA-H 16	Chinese Hamster/Human	16
RA 17	Mouse/Human	17

In addition, there are six other hybrids in which identity of the human chromosome is not confirmed.

6. In parallel to these studies we have developed a method of DNA fingerprinting using PCR based DNA amplification for the identification of different human chromosomes. DNA fingerprinting allows the identification and mapping of subchromosomal fragments present in different hybrids.

Papers in Preparation

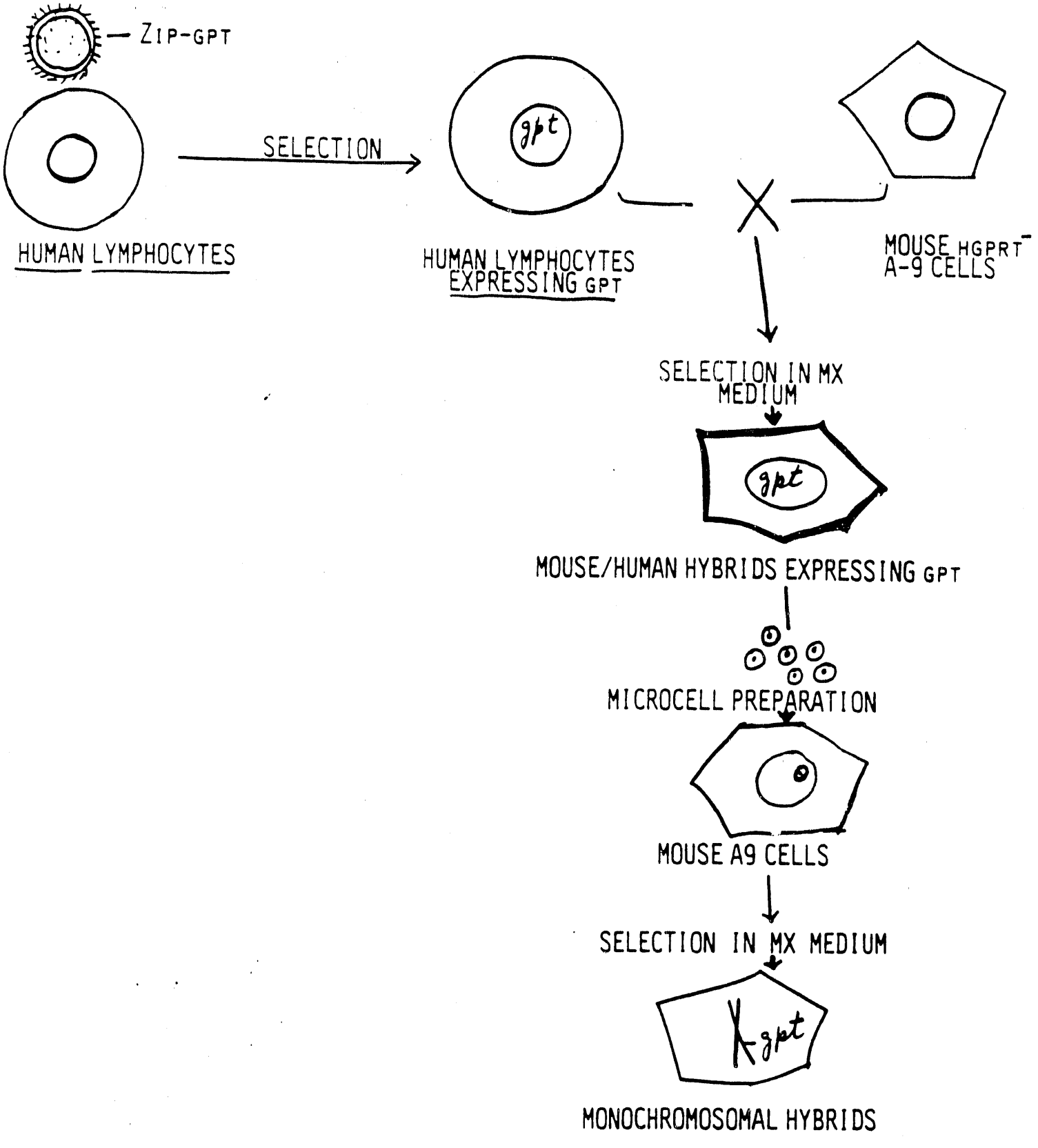
1. Sidhu, M.S., B. Helen and R.S. Athwal. Selective DNA Amplification: A method for chromosome fingerprinting and identification. (Prepared for publication in *Proceedings of National Academy of Sciences*, U.S.)

2. Sidhu, M.S., A.K. Sandhu, F. Chen and R.S. Athwal. PCR amplification and cloning of DNA segment flanking an integrated marker: Identification of chromosome carrying the selectable marker in human cells. (Prepared for publication in *Proceedings of National Academy of Sciences*, U.S.)

3. Helen, B., P. Kaur and R.S. Athwal. Microcell mediated transfer of individual human chromosomes to rodent cells: Production of monochromosomal hybrids.

Fig 1

EXPERIMENTAL APPROACH TO PRODUCE MOUSE/HUMAN MONOCHROMOSOMAL HYBRIDS



DOE F538 (5-86)

OMB Control No. 1910-1400

U. S. DEPARTMENT OF ENERGY

NOTICE OF ENERGY RD&D PROJECT

1. Descriptive TITLE of work (150 characters including spaces)

Monochromosomal Hybrids for the Analysis of Human Genome

2. CONTRACT or grant number DE-FG02-89ER60866

2A. MASTER contract number (GOCO's)

2B. Responsible PATENT office

3. Performing organization CONTROL number (internal)

3A. Budget and Reporting code KP0404

3B. Funding YEAR for this award 1991-1992

4. Original contract start date July 1, 1989

4A. Current contract start date February 1, 1991

4B. Current contract close date January 31, 1992

4C. Anticipated project termination date January 31, 1995

5. Work STATUS

☐ Proposed ☒ Renewal

☐ New ☐ Terminated

5A. Manpower (FTE)

5B. CONGRESSIONAL district 10th

5C. STATE or Country where work is being performed New Jersey USA

5D. COUNTRY sponsoring research

6. Name of PERFORMING organization University of Medicine and Dentistry of New Jersey-New Jersey Medical School

6A. DEPARTMENT or DIVISION	6B. Street Address	6C. City, State, Zip Code
Microbiology and Molecular Genetics	185 South Orange Ave.	Newark, New Jersey 07103-2714

7. Circle only one code for TYPE of Organization Performing R&D:

CU - College, university, or trade school
FF - Federally funded RD&D centers or laboratory operated for an agency of the U. S. Government
IN - Private industry
NP - Foundation or laboratory not operated for profit
ST - Regional, state or local government facility
TA - Trade or professional organization
US - Federal agency
XX - Other
EG - Electric or gas utility

8A. Contractor's PRINCIPAL INVESTIGATOR/s or project manager Name/s (Last, First, MI) Athwal, Raghbir S.

8B. PHONE/s (in order of PI names with commercial followed by FTS)

Comm. 201-456-5215 ; FTS ; Comm. ; FTS

8C. PI/s address (if different from that of Performing Organization)

9. DOE SUPPORTING Organization (DOE Assistant Secretary and office sponsoring the work; technical monitor; and administrative monitor).

9A. PROGRAM division or office (full name) Office of Health and Environmental Research — ER-72 (Program Office Code)

9B. TECHNICAL monitor (Last, First, MI) Barnhart, Benjamin J.

9C. Address Office of Health & Environ. Res., Office of Energy Research, U.S. Dept. of Energy, Washington, DC 20585

9D. Phone Comm. (202) 353-3683 FTS 233-3683

9E. ADMINISTRATIVE monitor (Last, First, MI) _____

10. FUNDING in thousands of dollars (K$). Funds represent budget obligations for operating and capital equipment (FY runs October 1 – September 30).

Funding organization(s)	Current FY ____	Next FY ____
A. DOE		
B.		
C.		

10D. Does the current FUNDING cover more than one year's work? Yes ____ No X

E. If yes, provide dates (from when to when). _____

11. Descriptive SUMMARY of work. Enter a Project Summary using complete sentences limited to 200 words covering the following: Objective(s), state project objectives quantifying where possible (e.g., "The project objective is to demonstrate 95% recovery of sulphur from raw gas with molten salt recycling at a rate of one gallon per minute."); approach, describe the technical approach used (how the work is to be done); expected product/results, describe the final products or results expected from the project and their importance and relevance.

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12. PUBLICATIONS available to the public. List the five most descriptive publications that have resulted from this project in the last year that are available to the public. (Include author, title, where published, year of publication, and any other information you have to complete full bibliographic citation.) Use the back of this form or additional sheets if necessary.

1. Sidhu, M.S., B. Helen and R.S. Athwal. Selective DNA Amplification: A method for chromosome fingerprinting and identification. (Prepared for publication in Proceedings of National Academy of Sciences, U.S.)

2. Sidhu, M.S., A.K. Sandhu, F. Chen and R.S. Athwal. PCR amplification and cloning of DNA segment flanking an integrated marker: Identification of chromosome carrying the selectable marker in human cells. (Prepared for publication in Proceedings of National Academy of Sciences, U.S.)

3. Helen, B., P. Kaur and R.S. Athwal. Microcell mediated transfer of individual human chromosomes to rodent cells: Production of monochromosomal hybrids.

13. KEYWORDS (Listed five terms describing the technical aspects of the project. List specific chemicals and CAS number, if applicable.)

Mouse/Human Monochromosomal Hybrids

Microcell Fusion

Chromosome Transfer

14. RESPONDENT. Name and address of person filling out the Form 538. Give telephone number, including extension (if you have FTS number, please include it) at which person can be reached. Record the date this form was completed or updated. The information in Item 14 will not be published.

Respondent's Name: Raghbir S. Athwal, Ph.D. Phone No.: 201-456-5215 Date:

Street: 185 South Orange Avenue

City: Newark State: New Jersey Zip: 07103-2714

15. Additional space for furnishing information in items 1 to 14. (Indicate item numbers to which answers apply.)

Item No.	

NOTICE: Return this form to the office indicated in the reporting requirements for your award agreement covering this project. If you have completed a similar programmatic office project description during the current Fiscal Year, complete only the new data elements on this form and send it and a copy of the description completed earlier to Department of Energy, Office of Scientific Information, P. O. Box 62, Oak Ridge, TN 37831.

END

DATE FILMED

02/27/91