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ISOLATION OF cDNAs FROM THE HUMAN X CHROMOSOME AND DERIVATION OF RELATED STSs

Progress Report

May 1992 — December 1992

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A. Progress 4/1/92-12/31/92

One of the major aims of this application involved the further characterization of ESTs generated by the Venter group and assigned to the X chromosome. Toward this aim, a number of cDNAs are currently under study. Seventeen ESTs assigned to the X have been collected along with associated cDNAs when available. PCR primers for 14 of these have been synthesized and used to sub-localize the genes using a somatic cell hybrid mapping panel. Five sub-assignments have been made and several more are in progress. Cosmid and YAC clones have been identified from arrayed libraries for several of the probes or STSs respectively. DNA sequence analysis of nine of the cDNA clones is underway focussing on the sequences at the opposite end of the insert from the EST sequence. Full-length sequencing of each clone is the goal, as well as validation of the clone's integrity through isolation of overlapping cDNAs from additional libraries.

An eighteenth cDNA clone is undergoing analysis as well. This clone was derived from a brain cDNA library in the laboratory of Dr. Ben Oostra at Erasmus University in Rotterdam, and has the peculiar feature of showing multiple loci on the X by in situ hybridization analysis. This clone is known as H10, and its full length sequence is nearing completion. More than one dozen cosmid clones have been isolated from a flow-sorted X chromosome library, and the localization of these is in progress. The following table summarizes the results for these eighteen novel cDNAs:

DXS#	Primers	cDNA	Sublocalized	Cosmids	YACs	Sequence
----	----	H10	multi?	13		~800 bp

1004E	10158	HFBCG24	p11.21-p21.2	6		~250 bp
	10159					
1005E	10152		in prog.			
	10153					
1006E	10156		p11.23-q21.2			
	10157					
1007E	10150		in prog.		in prog.	
	10151					
1008E	10148		in prog.		in prog.	
	10149					
1009E	10160	HHCMB03	q26-q27.1	12		
	10161					
1010E	10154	HHCPA14	q28 (L1-RCP)	3	in prog.	
	10155					
1011E	10162	HHCPA83	p11.23-q21.2			~250 bp
	10163					
1012E	10146					
	10147					
1013E	10164	HFBBA54				~250 bp
	10165					
1014E	10144					
	10145					
1015E	10166	HFBCA37				400 bp
	10167					
1016E	10168	HFBCC15			in prog.	400 bp
	10169					
1103E	10170	HFBCD27				400 bp
	10171					
(D0S) 1424E		HFBCC06				in prog.
1456E		HFBCC55				in prog.
1522E		HFBCD88				in prog.

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Derivation of additional X-linked cDNAs is another major aim of the study. Less progress has been made in this area. Efforts to use somatic cell hybrids to derive additional X-linked cDNAs have not yet commenced.

A method for identifying genes present in previously isolated YAC inserts is being developed. This involves adaptation of an exon-trapping method developed in the laboratory of Dr. Susan Berget at Baylor to scan YAC inserts. A novel "targetting" vector is being built with the aim of placing the exon trapping vector into YAC inserts via homologous recombination into Alu sequences found in human inserts. The vector will confer the dominantly selectable hygromycin resistance gene to the YAC-bearing yeast cell, providing for isolation of recombinants. Inserts into the Alu sequences will bring the trapping vector in proximity to human genes for testing via electroporation of the YAC sequences into mammalian cells. It is likely that these sequences will be presented to the mammalian cell as a complex pool of YAC inserts each with one or more vector insertion. While the hygromycin gene could be used for isolation of transfected clones, it is anticipated that most of these studies will utilize transiently transfected populations of cells.

The exon-trapping vector specifically recognizes 3' terminal exons, requiring both splice acceptor and poly adenylation signals to provide a signal. Any intervening internal exons are also trapped by the vector. A major advantage of this system is the ability to trap adjacent exons, not simply those between two vector portions. This is accomplished through the use of RT-PCR using primers directed to poly A sequences and to vector sequences within the transcript. Results from the Berget laboratory suggest that the trapping method can identify quite distant 3' exons (>20 kb), thus the region scanned by each insertion can be substantial. The vectors and methods are designed to be useful in the AB1380 yeast cell background common to the majority of YAC libraries. Initial vectors are currently being tested, and it is anticipated that first feasibility studies will be undertaken in the next reporting period.

B. Objectives 1/1/93-12/31/93

No major alterations from the proposed studies are contemplated. Major efforts will concentrate on the eighteen novel X-linked genes under study, with the expectation that each will be localized to a well-defined region of the chromosome and in most cases to YAC and cosmid contigs. In addition, each cDNA insert available will be sequenced in its entirety, allowing comparison to known DNA and peptide sequences. Additional cDNAs will be sought for many of these ESTs in order to confirm the integrity of the initial clones and their sequences.

The development of the exon trapping system will receive greater priority in the coming period, as it is clear that the entire X chromosome will soon be available as a minimal set of overlapping YAC clones, and that the next challenge will be efficient means for cDNA identification from cloned genomic DNA.

It is expected that the next year of funding will generate significant new insights into the organization of the human X chromosome and associated genes while continuing to produce cloned materials useful for these analyses.

C. Publications 4/1/92-12/31/92

No publications have resulted from this effort as yet.

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