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Our research interest is focused on the identification of genes from chromosome 17. The isolation of genes transcribed from chromosome 17 will provide candidates for the proposed sporadic breast cancer genes and genes for other human disorders.The ability to isolate genes in a chromosome specific manner provides simultaneous identification of the expressed sequence and a chromosomal location. Our approach identifies expressed sequences by reciprocal probing of arrayed cDNA libraries and a chromosome specific cosmid library. To date from the cDNAs isolated from human chromosome 17 we have identified two very important genes. One gene, which encode a coactosin like protein (*CLP*) and maps to 17p11.2 has been demonstrated by us and our collaborators to be involved in the Smith-Magenis Syndrome, a neuro-muscular disorder. A second gene which encodes a putative transcription factor has been demonstrated by my laboratory to be a key gene in the mammalian circadian rhythm pathway. Based on protein homology, a second circadian gene was recently identified by my laboratory .

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## Introduction

The role of genetics in cancer is now well established with the identification of several genes where the presence of mutation has been associated with cancer formation (1). It has been shown that mutations of tumor suppressor genes, as negative regulators of cell division, contribute to oncogenesis by interference with mechanisms restraining cell multiplication. Thus genes encoding proteins involved in cellular functions including signal transduction, transcription and phosphorylation/dephosphorylation cell cycle pathways are prime candidates for tumor suppressor genes. In familial forms of cancer, a combination of germ-line and somatic mutations on each allele results in chromosome loss or deletion, meiotic recombination, or gene conversion. Similar events uncovering recessive somatic mutations also occur in sporadic forms of cancer. The loss of genetic material inherited from one parent can be detected by loss of heterozygosity (LOH) analysis using genetic markers. LOH studies on tumors and linkage analysis in inherited forms of cancers have resulted in identification of several tumor suppressor genes (2). The gene for hereditary breast cancer, namely BRCA 1 is also associated with hereditary cancer of the ovary (2,3). The gene for BRCA 1 was mapped to chromosome 17q21, a region that is also associated with allele losses (loss of heterozygosity, (LOH)) in sporadic breast and ovarian cancer (4,5,6,7,8,9). After the initiation of our studies to clone BRCA1, the gene BRCA 1 was identified by the Scolnick group (10) via positional cloning methods. Many mutations in BRCA 1 gene were found in patients with hereditary breast and ovarian cancer (11,12). Surprisingly, these studies also show that mutations in BRCA1 are rare in sporadic breast and ovarian cancers that are thought to be due to susceptibility to the disease at this locus (13,14,15). Together, the LOH studies and the lack of mutation in BRCA 1 have led to the proposal that there is another gene within this region of 17q12-q22 that is associated with sporadic breast and ovarian cancer in women (16). Many studies have demonstrated that LOH in other regions of human chromosome 17 is associated with breast cancer (17,18). These studies indicate that a region telomeric to the P53 gene at 17p12-17p13.3 (about 3 cM) is believed to harbor a separate tumor suppressor gene associated with breast cancer. The strategies for cloning a disease-related gene included either functional or positional cloning approaches (19). With the effort of the Human Genome Initiative in cDNA and expressed sequence tag (EST) mapping, a candidate gene approach to finding human disease genes has been predicted to be the future trend. Our research interest is focused on the identification of genes from chromosome 17 based on the reciprocal probing approach (20). The isolation of genes transcribed from chromosome 17 will provide candidates for the proposed

sporadic breast cancer genes and genes for other human disorders. To date from the cDNAs isolated from human chromosome 17 we have identified two very important genes. One gene, which encode a coactosin like protein (*CLP*) and maps to 17p11.2 has been demonstrated by us and our collaborators to be involved in the Smith-Magenis Syndrome, a neuro-muscular disorder (21). A second gene which encodes a putative transcription factor has been demonstrated by us to be a key gene in the mammalian circadian rhythm pathway (22). This gene maps to 17p12 which we have named *Rigui/mPer1.* This discovery for the first time allowed scientist to follow the mammalian clock at a molecular level and provide an unprecedented window in to its working mechanism. Understanding the human biological clock could have many potential benefit. In addition to the understanding of the human sleep-wake cycle, it could also generate strategies to develop therapies for many disorders including shift work disorder, depression, sleep disorders, and seasonal affective disorders. Molecular studies into the hypothesis that patients responsiveness to chemotherapy display circadian patterns could now be address.

## Results

We have reported a method for the isolation of chromosome specific cDNAs using high density arrayed cDNA and chromosome specific cosmid libraries (20). The ability to isolate genes in a chromosome specific manner provides simultaneous identification of the expressed sequence and a chromosomal location. This technology identifies expressed sequences by reciprocal probing of arrayed cDNA libraries and a chromosome specific cosmid library.

The isolated chromosome specific cDNA clones were sequenced through one pass sequencing from the 5' and 3'ends. The corresponding cosmids were used for *fluorescent in situ hybridization* (FISH) mapping to localize their chromosomal position. The sequence information was used to generate sequence tag site (STS) primers for polymerase chain reaction (PCR) mapping on chromosome 17 somatic hybrid cell-lines to further confirm the cDNA and the corresponding cosmid map position.

In our last report, we proposed to change strategy for the final year of this grant. The new strategy include the studies of human genes with trinucleotide repeats and the isolation of genes that may encode for regulatory proteins. These change in strategies has resulted in major discoveries of genes involved in human diseases and very important biological functions.

## Identification of Mammalian Circadian Rhythm Gene

Biological rhythms are a fundamental characteristic to living systems. In response to daily environmental cues, the physiology and behavior of all living organism from bacteria to humans are controlled by circadian rhythms driven by endogenous oscillators. Although the behavioral and physiological properties of the mammalian circadian rhythms are well documented, the molecular and genetic mechanism are unclear. In *Drosophila*, two genes, *period* and *timeless*, are essential components of the circadian clock. A heterodimer of Per and Tim protein is thought to regulate the circadian process by creating a negative feedback loop controlling per and tim expression. However, in the decade since per was first found from *Drosophila* melanogaster, no mammalian *per* homolog had yet to be reported.

Using reciprocal probing between cDNA and cosmid libraries, we have identified a human chromosome 17 specific transcripts that encodes a bHLH-PAS protein domain with significant sequence similarity to Drosophila melanogaster Period. Overall protein homology (identical amino acid and conserved + neutral substitution) is 44%. We have named this gene RIGUI (after an ancient Chinese sundial) or mammalian *period* (*mPer1*) gene.

*Per* and *tim* in Drosophila, circadian oscillator genes, are expressed in a periodic manner during the 24 h day/night cycle. To examine whether expression of *RIGUI /mPer1 behaves* in a similar way, we studied its expression in mice. A murine brain cDNA library was screened with the human *RIGUI /mPer1*4.7 cDNA as probe, and a mouse homolog termed *mrigui/mPer1* was identified. RNase protection assay using mouse retinae RNA collected every 4 hours during a 12 h light/ 12 h dark cycle showed that *m-rigui /mper1* mRNA level increased during the light phase and decreased during the dark phase.

*In situ* hybridization demonstrated that *m-rigui /mper1* mRNA is expressed in the suprachasmatic nucleus (SCN), the master regulator of circadian rhythms in mammalian systems. Expression was the highest at ZT6 (whereby Zeitgeber time ZT0 is when lights were turned on and ZT12 is when lights were turned off) and the lowest at ZT18. We further observed the circadian pattern of expression in Purkinje neurons of the cerebellum and *pars tuberalis*, but expression patterns are shifted in phase from that of SCN. Expression analysis by *in situ* hybridization also revealed that circadian expression in the SCN is sustained in constant darkness, and a shift in the light/dark cycle evokes a proportional shift of *m-rigui* expression in the SCN. These are properties expected of a mammalian circadian genes. To date as far as we know, this is the first mammalian gene that fulfills these circadian rhythm properties. Taken together, the sequence homology and circadian patterns of expression suggest that *RIGUI/mPer1* is a mammalian ortholog of *Drosophila period* gene, raising the possibility that a regulator of circadian clocks in mammals have been identified. (This work was published in the Journal *Cell* and was presented as a late breaking news

in the American Society of Human Genetics meeting in Washington, D.C. October 27-November 1, 1997). The discovery of *Rigui/mPer1* was ranked by the journal *Science* (which is the most influential scientific magazine in the USA) as one of the nine runners-up in 1997 Scientific Break-Through of the Year (see December 19, (1997) issue of *Science*).

The discovery of *Rigui/mPer1* rapidly lead to the discovery of a second circadian gene *mPer2* by my laboratory. The new gene has many similar properties that was described for *mPer1*. The discovery of *mPer2* was recently published in the journal *Cell* (Albrecht *et al., Cell, 91,* 1055-1064 (1997)).

**A gene involved in the Smith-Magenis Syndrome**

Our present studies also identified a gene that encodes for a coactosin like protein (*CLP*) and maps to 17p11.2. Based on mapping studies we have been able to linked its involvement in the Smith-Magenis syndrome (SMS). Smith-Magenis syndrome is a contiguous gene deletion syndrome with a frequency of 1/25,000 live births. The clinical findings of SMS include mental retardation, neurobehavioral abnormalities, sleep disturbances, short stature, minor craniofacial and skeletal anomalies, congenital heart defects and renal anomalies. This wide spectrum of phenotypic variation could have arise from a variation in the size of the DNA deletion. Our study shows that the *CLP* gene is duplicated in a repeat domain containing a gene cluster flanking the SMS deletion region. We were able to demonstrate that the *CLP* gene flanks this repeat domain and that the duplication of this repeat leads to a recombination of the repeat domain resulting in the loss the of chromosomal region missing in SMS patients. (A paper describing this work was published in Nature Genetics (Chen et al., *Nature Genet.* **17,** 154-163 (1997)).

## Identification of the gene that causes spinocerebellar ataxia type 6 (SCA6).

Hereditary cerebellar ataxias are a clinically and genetically heterogenous group of neurological disorders associated with dysfunction of the cerebellum and its afferent and efferent connections. To date, seven autosomal dominant spinocerebellar ataxias (SCAs) have been mapped to human chromosomes 6, 12, 14, 16, 11, 19 and 3 with the loci designated as SCA1, SCA2, SCA3, SCA4, SCA5, SCA6, and SCA7, respectively. Except for SCA5, the genes responsible for these SCAs have been identified. A common genetic mutation involving the expansion of CAG trinucleotide repeat sequences has been shown to be the cause of these SCA

disorders. In addition to the SCA disorders, CAG repeat expansions have also been associated with Huntington disease [HD], spinobulbar muscular atrophy [SBMA], and dentatorubral-pallidoluysian atrophy/Haw-River syndrome [DRPLA/HRS]. A common feature among these disorders is the observation that the CAG repeats in the respective genes are located in the coding regions and are translated into polyglutamine tracts in the protein product. Polyglutamine tract expansion is central to the progressive nature of these diseases which results in the degeneration of neurons of the central nervous system. Typically, the CAG repeats in the respective gene shows length polymorphism in the human population and is generally less than 40 repeat units. In the affected individuals, the CAG repeat is expanded beyond the normal size range. Intergenerational changes of the CAG repeat size are often biased towards further expansion, particularly if paternally transmitted, providing the molecular basis of genetic anticipation . To date, these generalized observations are applicable to all the polyglutamine disorders with some variation in specific diseases with respect to the length of the repeat and its effect on the disease state. Most unusual in this regard is SCA6, where the expansion of the mutant alleles are relatively modest, in the range of 21-30 repeats, which are much smaller than the expanded alleles seen in any of the other CAG repeat diseases and are well within the normal range of polyglutamine tracts seen at the other loci in many unaffected individuals. Furthermore, intergenerational changes of the CAG repeat length is relatively rare in SCA6 families.

The gene for SCA6 was identified by screening human brain cDNA libraries with a $(CTG)_7$ repeat oligonucleotide probe. A cDNA containing a polymorphic CAG repeat sequence belonging to the $\alpha_{1A}$ voltage-dependent calcium channel gene (CACNL1A) was isolated during this screen. Using a large scale genotyping survey strategy with this polymorphic CAG repeat and DNA samples from patients with late onset neurodegenerative diseases, the CAG repeat sequence in human $\alpha_{1A}$ voltage-dependent calcium channel gene was initially found to be expanded in eight families diagnosed with autosomal dominant cerebellar ataxia. Our studies suggested that an expansion of a CAG repeat leading to an increase in polyglutamine length in certain isoforms of the human $\alpha_{1A}$ voltage-dependent $Ca^{2+}$ channel protein is the primary cause of one form of cerebellar ataxia which we designated as spinocerebellar ataxia type 6 (SCA6).
( The manuscript describing the discovery of SCA6 was the 4th most cited paper during 1997 for manuscripts published in 1997. See Science **279**, p1639 (1998)).

## Conclusions

The original objective of this proposal was to identify the gene responsible for BRCA1 by selecting for genes specific for human chromosome 17. The discovery of the gene for BRCA1 after the initiation of this project has altered the goal of this proposal. The proposed goals for the past year focused on detailed characterization of genes identified from human chromosome 17 have yielded important discoveries. Four very important genes, two of which are linked to human disorders and two which may be central to the human circadian pathway were discovered in my laboratory. Without this funding from the Department of Defense, the ability to make these discoveries would have been severely compromise.

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