

A 1.5-Mb PAC/BAC Contig Spanning the Prader-Willi Syndrome Critical Region (PWCR)

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Prader-Willi syndrome (PWS) is a multiple anomalies/mental retardation syndrome. The putative PWS gene(s) remains unknown, and its occurrence is based on genomic imprinting at chromosome 15q11-q13. We have constructed a 1.5-Mb, fine, physical map of PWS critical region (PWCR) between two markers, *DI5S9* and *DI5S174* at 15q11-q13. The map is composed of 32 PAC and 3 BAC clones without any gaps. By the PAC/BAC-end sequencing procedure, a total of 26 sequence tag site (STS) markers were newly generated, and 5 expressed sequence tags (ESTs) were mapped in the region. The contig map was verified by both STS and fluorescence in situ hybridization analyses. Our map has higher resolution, compared with a previous YAC-based map of PWCR. It is useful for further genome analysis, especially on genomic imprinting of this region.

Key Words: Prader-Willi syndrome critical region (PWCR); 15q11-q13; imprinted region; PAC/BAC contig; physical map

Introduction

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct mental retardation disorders with parent-of-origin-specific deficiency on chromosome 15q11-q13. PWS is caused by either a deletion (about 70% of pa-

tients) of 15q11-q13 region on paternally derived chromosome, maternal uniparental disomy (25%), or an imprinting center defect (<5%).^{1,2)} In contrast, AS is caused by either a deletion (70% of patients) of the same region on the maternally derived chromosome 15, paternal uniparental disomy (5%), an imprinting center defect (<2%), a single gene mutation in *UBE3A* (<5%),^{3,4)} or unknown defects (20%). These findings indicated the existence of imprinted genes in the common deleted region, especially in an interval between *DI5S9* and *DI5S174*; this region is further referred to as Prader-Willi syndrome critical region, PWCR. Indeed, the 15q11-13 region contains a cluster of imprinted genes, i.e., paternally expressed *ZNF127*, *NDN*, *MAGEL2*, *SNRPN* and *IPW*, and maternally expressed *UBE3A*.^{3,5-10)} Some additional imprinted genes may still exist in this region. Although it is most likely that PWS is a contiguous gene syndrome,¹¹⁾ the role of paternally expressed genes in PWS remains unknown. Thus, a fine physical map of PWCR is of great importance to isolate putative, additional imprinted genes. A complete physical map for PWCR with a YAC clone contig,¹²⁻¹⁶⁾ and a higher resolution map based on PAC/BAC clones have been constructed.¹⁷⁾ However, these maps may be difficult to be used for further genome analysis, since the YAC-based map is less fine and the PAC/BAC-based map had at least three gaps within PWCR.

This study is to construct another fine map of PWCR, composed of P1-derived artificial chromosome (PAC) and/or bacterial artificial chromosome (BAC) clones.

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Materials and Methods

Screening of PAC and BAC clones, generation of novel sequence tag sites (STSs)

A PAC library¹⁸⁾ that was arrayed in 384 wells with a 5-fold genome coverage was used as a primary screening source. PAC clones were identified by PCR amplification of DNA pools, and provided as a framework for walking and gap-filling as described previously.¹⁹⁾ The library was screened initially with five known STSs, each corresponding to *D15S9*, *D15S11*, *D15S13*, *D15S63* or *D15S174* locus. Among them, *D15S9* and *D15S174* are most telomeric and centromeric known markers within PWCR, respectively, and a physical distance between the two is estimated to be ~1.5 Mb.¹⁵⁾ PCR was performed in a 20 μ l mixture containing 10 ng of clone DNA, 1 \times Perkin Elmer Buffer II, 200 μ M of each dNTP, 1 μ M of each primer, 1 unit Amplitaq (Perkin Elmer/Cetus, USA) in Thermal Cycler (Perkin Elmer 9600 or 9700) under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 30 sec, at 45°C - 60°C for 30 sec and at 72°C for 30 sec, and final extension at 72 °C for 5 min. After DNA was prepared using Plasmid Midi kit (Qiagen, USA) from individual PAC clones isolated, DNA (2 μ g) of their insert ends was sequenced using Cy5 Cycle Sequencing kit (Pharmacia Biotech, Sweden) and ALFexpress DNA sequencer (Pharmacia Biotech). Sequences (5'-3') of Cy5-labeled T7 and SP6 primers were AATACGACTCACTCACTATAG and ATTAGGTGACACTATAG, respectively. Crude PAC clones difficult to be sequenced were subcloned into pBluescript SK II(+) by plasmid rescue and then sequenced. To fill contig gaps, a CITB-BAC library²⁰⁾ purchased from Research Genetics (USA) and a IRB-BAC library (RPCI-11) were screened with the same procedure as in the PAC library screening. In addition, DNA of two YAC clones (y269-D-12 and y333-A-10, kindly provided by Dr. Ledbetter)¹⁵⁾ was examined on whether they cover STSs in PAC/BAC clones.

Fluorescence in situ hybridization (FISH)

BAC/PAC-DNA that was labeled with biotin-16-dUTP (Boehringer-Mannheim, Germany) by nick translation was hybridized to normal metaphase chromosomes, together with human Cot-1 DNA (GIBCO-BRL, USA) to suppress repetitive sequences, as described previously.²¹⁾ FISH signals were detected with FITC-conjugated avidin (Vector Laboratories, USA). Photomicroscopy was performed under a fluorescence microscope using two filter combinations, G-2A and B-2A (Nikon, Japan).

Search for expressed sequence tags (ESTs) in the contig

Based on the Human Transcript Map,²²⁾ 17 EST markers supposed to be mapped to a region between *ZNF127* and *SNRPN* were collected, and were tested by PCR whether they hit PAC/BAC clones in the contigs.

Results and Discussion

Construction of a PAC/BAC contig

We first constructed four short PAC contigs using the five known STSs, *D15S9*, *D15S11STS*, *D15S13*, *D15S63* and *D15S174*. As a result, three gaps remained, such as those distal to *D15S11CA*, *D15S1524* and to *D15S13*. Despite of much effort to screen the PAC library repeatedly, one gap distal to *D15S11CA* remained. We then screened the two BAC libraries using STSs flanking the gap, and identified BAC clones to fill it (Fig. 1). FISH confirmed that all PAC and BAC clones isolated are assigned to 15q11-q13. Finally, we have successfully constructed a ~1.5-Mb fine physical map (PAC/BAC contig) encompassing two markers, *D15S9* and *D15S174*. The map consists of 32 PAC and 3 BAC clones, representing one (around clones 984-A-13, 392-P-8, and 169-P-23) to eight (at 167T7 locus) clone coverage (Fig. 1). The extent of the contig was estimated, based on the previous data for YAC and for a PFGE map this region.¹⁵⁾ The contig map was verified by both STS and FISH analyses, and has higher resolution, compared with the previous YAC-based map.¹⁵⁾ There has been a PAC/BAC-based map constructed for a region from *D15S18* (*IR39*) to *D15S1019*,¹⁷⁾ but it retained three gaps at regions around *D15S9*, between *D15S11* STS and *D15S13* STS, and between *D15S13* STS and *D15S63*. Our map has no such gaps and covers all these regions.

STS and EST mapping

A total of 26 novel STSs were generated at PWCR by direct sequencing of the ends of PAC/BAC clones (Table 1, Fig. 1). A search for ESTs through the Human Transcript Map²²⁾ revealed that five new ESTs (*WIAF-831*, *sts-H58001*, *SGC44643*, *R99003*, and *WI-15028*) hit either of the PAC/BAC clones between *D15S9* and *D15S174*. The search also confirmed that five known genes (*ZNF127*, *NDN*, *MAGEL2*, *SNRPN* and *IPW*) are located to the region.

As in another imprinting domain at 11p15.5,²³⁻²⁶⁾ there is a cluster of imprinted genes in PWCR between

DI5S9 and *DI5S174* loci, i.e., five paternally expressed (maternally imprinted) genes (*ZNF127*, *NDN*, *MAGEL2*, *SNRPN*, and *IPW*). Although we have mapped the five ESTs (*WIAF-831*, *sts-H58001*, *SGC44643*, *R99003*, and *WI-15028*) in this region, no genes/ESTs could be as-

signed in intervals between *MAGEL2* and *DI5S1524* and between *DI5S1524* and the *SNRPN* region (Fig. 1). It thus remains to be investigated whether the regions are truly geneless. This is of great importance to know the genomic imprinting mechanism in PWCR.

Table 1. Twenty-six novel STSs generated from PWCR

STS	Primer sequences		Ta(°C)	Size(bp)
491T7	ATAACATCAATGCCATTTTCAGC	AGTTCACATGGTTACCTCTGGG	60	119
491SP6	AACATACCAGAATGCCCAGC	CAGAACTTTGGGAGGCTGAG	60	312
75T7	CAGTAATCCTATGGAGGCTTG	AAAACGTGCCACTAGTAGGC	57	256
437T7	AAAAGGCAACACAAACCACC	GAGCTGTTTGTAAATTGGTCCC	60	242
205T7	TTGAATGGAGGGATGGATATG	ACGTTGTGTGTGTGAAAGTCC	60	224
984T7	TTCATGTGAGGAAAAGGTATGC	TCACAGCATGCCAAGATATAGG	54	175
984SP6	CCTGATGTGATTGGGTTAGACC	TTGATGGATGATCTCTCCTGG	56	182
247SP6	CATTTTGCCTTATTATCGTG	CACCTACTCTCCTATTTCAA	50	343
206T7	ACAAAGTGGCTAATGGAAGA	GAGAACAAAAACAAAGGTAT	45	145
206SP6	TTCTCCTATCCTTCTCTAAAA	CCATATGCCCCCTCCTCTGAC	50	173
332T7	TTGGAGGATGAGTTGAGGTC	GTAGGTGTTAGTTTGAGCA	47	117
392T7	GAAAAGTCTTTCCAACCCTGG	TCACCCCTGTCGAAACAGTG	60	102
392SP6	CACAATTCTGCATTCAAGGG	GGCAACAAGAGAGAACTCCC	60	120
420T7	CATGAGTTTGTGAGGAGAGCC	TGTGGCTCTATCTGGGCAG	60	237
420SP6	TTCTATGTTCTGTGATTATAGGAG	CTGAATCAACTGTAACATTTTATC	52	216
488T7	TTTCCTTCTATCACTTATTG	TCTGTTTCTCTCATTTCTTA	45	249
488SP6	AGAATGAGTGACAGGAAAGG	TCAAAGTACACTGCTTTCATGC	54	173
914T7	TAGAATGAAACACATGAAAAGG	GAAGGCTCACAAATGTGTAC	56	396
33SP6	CCTCCAATATATGATAAATCTAGAC	GACTACAAGGAAAAACTTGACAC	53	186
300SP6	CAAATATGGTTTGGACATGCC	CTTGACACAATCTTAGGGCTCC	60	185
84T7	CGTATATGATTATATATGCATAAGG	CTTTGTCCCTTGTATC	47	118
84SP6	AGAGCATACAGAGGTGACTC	TTGGCTTAGTGAGTCTAGG	53	125
167T7	CAGTTCTCTGGCCTTCTTGG	GTAAAGAGTGGGCCTTGCTG	60	141
167SP6	CCTGTAATCCCAGTACTCAGG	TTTAGCCGCCGTACTTG	60	253
169T7	ACCCTGTCCCTACTAAAAATAC	GCTAGTCTATGTAGTTTGCATG	52	302
65SP6	TGTTGGTAGAGGGCCAGG	TACAGGCACCTGTACCAC	58	172

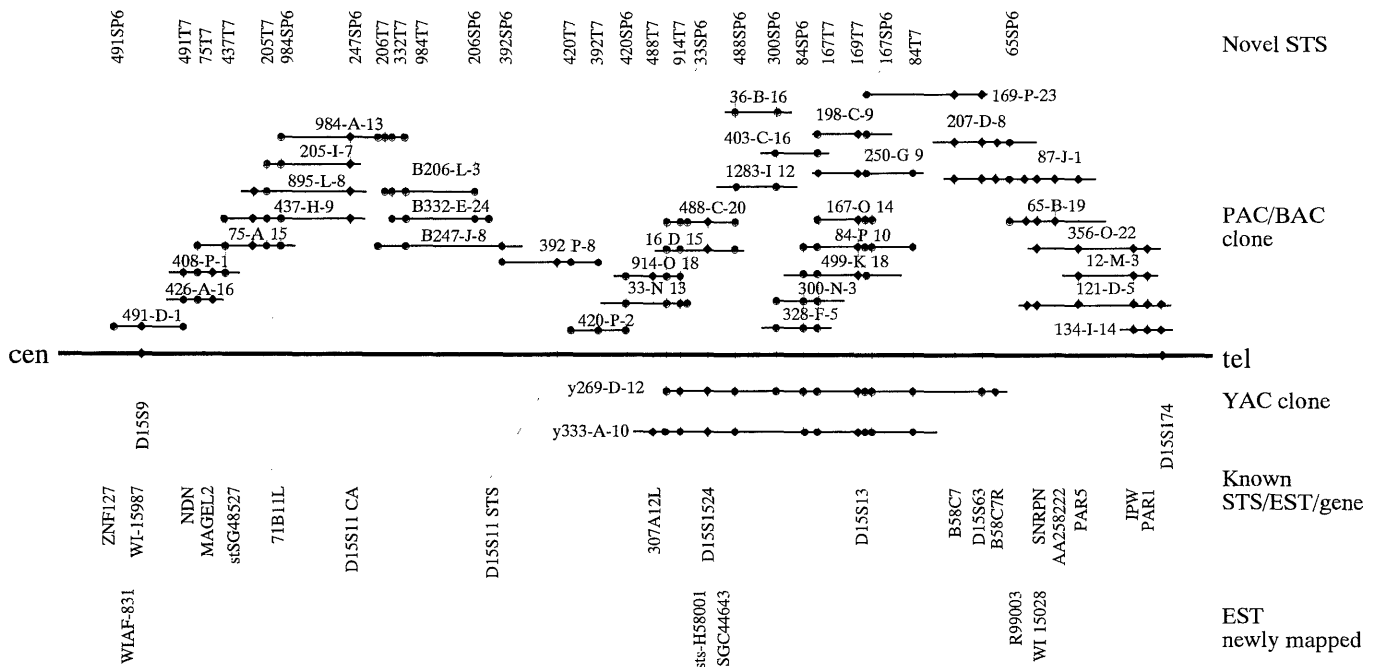


Fig. 1. A PAC/BAC contig encompassing *D15S9* and *D15S174* with novel STSs, known STSs/ESTs/genes, and newly mapped ESTs.

The PAC/BAC contig constructed in the present study, along with the newly generated 26 STS markers, are a valuable source for such studies.

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