



**MICRODISSECTION AND MOLECULAR CLONING
OF EXTRA SMALL RING CHROMOSOMES OF
HUMAN**

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Doctor of Philosophy**

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January, 1998

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SUMMARY

The changes of the dosage of normal genes, either by addition or deletion, can result in phenotypic abnormality. Constitutional small ring chromosomes accessory to the normal diploid karyotype change the dosage of genes by partial trisomy and such rings have been found in individuals with abnormal development. These small ring chromosomes have been relatively poorly characterized. From most reported cases, the ring chromosomes were identified by fluorescence *in situ* hybridization (FISH) with specific-centromere probes. However, the exact origin of the euchromatin of these ring chromosomes cannot be determined by this procedure.

To precisely identify their origins and genetic content and therefore provide a better groundwork for genetic counselling, extra small ring chromosomes from nine normal or abnormal carriers have been characterized by a combination of microdissection, FISH and molecular cloning. With microdissection and reverse hybridization, the origin of six ring chromosomes have been identified as from chromosome 1 (one case), chromosome 4 (two cases), chromosome 8 (two cases), and chromosome 15 (one case). All these ring chromosomes included the centromeric region of their original chromosomes and in addition, the ring chromosome 1 contained the heterochromatic region of chromosome 1q12; one ring chromosome 8 contained 8p11 and ring chromosome 15 contained 15q11.

A ring chromosome 4 was found to contain the centromere and portion of band 4q31 by reverse hybridization. Further investigation with molecular cloning revealed that this ring also contained the 4p13-14 region. Using isolated cosmid DNA, a third ring chromosome 4 was identified which contained a segment from the centromere to band 4q12. Using the same method, a ring chromosome 15 was further confirmed to contain the euchromatin from the most proximal area of long arm of chromosome 15 (15q11). Microdissected DNA from this ring chromosome 15 was used for FISH to characterize two *inv dup(15)s*. One *inv dup(15)* was composed entirely of alphoid

centromeric heterochromatin and the other one probably included inverted duplication of Prader-Willi syndrome (PWS)/Angelman syndrome (AS) region (15q11→13). Two other ring chromosomes, a ring chromosome 1 and a ring chromosome 20, were also studied by microdissection and cloning of the products. However, this study was unsuccessful as the initial microdissected products were contaminated with chromosome 6 and chromosome 5, respectively.

The comparison of these results demonstrated that molecular cloning is an effective method in the characterization of extra small ring chromosomes. With this method, one small ring 4 was found to originate from three discontinuous regions of chromosome 4 and this allowed us to hypothesize a new mechanism of ring chromosome formation, i.e. ring chromosome may be formed from an initial large ring by subsequent interlocking, breakage and fusion occurring during cell division to generate a smaller ring.

Another type of gene dosage change is chromosome deletion which forms a partial monosomy and result in abnormality. Wolf-Hirschhorn Syndrome (WHS) is caused by a deletion of the band 4p16.3 and patients with this deletion have growth and mental retardation, characteristic facies and seizures. In the present study, a subtle interstitial deletion of 4p16.3 in a patient has been characterized with FISH. This small interstitial deletion of 4p16.3 contributed to the refinement of the critical deletion region of WHS.