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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 6 (4): 391-396, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.391.396

A Deletion is Associated with Cy Mutant Chromosome in *Drosophila melanogaster*

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ABSTRACT

The Cy mutation is the most commonly used dominant marker for the second chromosome balancers in *Drosophila melanogaster*, but little is known about molecular mechanism underlying the Cy phenotype. So, the study aimed at detecting the difference at DNA level between the curly stocks and the wild type by PCR amplification and sequencing. Here we combined genomic and cytological information to detect difference at DNA level between curly stocks and the wild type. Firstly, by sequence analysis a 102 bp deletion which locates the genomic interval between genes syt and daw has been found to be associated with the Cy mutant chromosome. Using the deletion as a marker, we detected genotypes of flies from different developmental stages in $Cy^{67.3}$: embryo, the first star larva, the second star larva, the third star larva and pupa. Results showed that all flies with genotypes BB and AA have been dead before adults. At DNA level these results provided a powerful proof for a conclusion that the curly maintains itself in a balanced lethal stock. In addition, a prediction suggests that two transcription binding sites which contain TAAT or ATTA, core recognition sequences of homeoproteins in drosophila melanogaster, are located in the region in which the deletion occurs. So, it is interesting for better understanding the molecular mechanism underlying curly phenotype.

Key words: Genomic interval, PCR, sequencing, curly wing, homeoprotein

INTRODUCTION

Curly (Cy) is a easily distinguishable dominant mutant wing character. The degree of curvature of the wings of Curly flies is variable from a slight upright bent of the wings at tips to an extreme roll of 360 degrees or over. Its Genetics was originally identified by Ward (1923). The gene for curly is usually lethal in homozygous form, meanwhile the homologue of its chromosome in the Curly stock also contains a recessive lethal, since under normal temperature, Curly maintains itself in a balanced lethal stock (Ward, 1923), but little is known about the molecular mechanism underlying the phenotype. So, it is very interesting in Drosophila genetic community to study on molecular nature determining Curly phenotype. Conventional mapping locates the Cy locus at 23A4-B2 (http://www.flybase.org). In previous papers, the Cy locus was mapped to 23A2-B2 (Spencer *et al*., 1982), 23B3-B8 (Ashburner *et al*., 1983), 23 B1-B2 and 23C (Littleton and Bellen, 1994), respectively. A γ-ray mutagenesis and genomic walk suggest that at least a portion of the Cy locus indeed resides in 23B1-23B2, proximal of syt gene (Littleton and Bellen, 1994).

With fully sequenced genome of drosophila melanogaster available (Adams *et al.*, 2000), by combination of genomic and cytological information we can detect the difference at DNA level between curly stocks and the wild type.

A 5548 bp genomic interval (2799970-2805518) and daw gene are just proximal of syt gene on genome (Fig. 2B). Syt has been reported to interact with several other synaptic proteins (Bennett *et al.*,1992; Leveque *et al.*, 1992; Petrenkao, *et al.*, 1991), plays a central role in calcium activation of neurotransmitter release (Littleton *et al.*, 1993). Daw gene is widely expressed in different tissues including wing disc in larva (Parker *et al.*, 2006; Serpe and O'Connor, 2006). Still, no paper ever reported that mutants of daw gene were associated with Curly phenotype. So, the genomic interval between genes syt and daw was first selected to investigate whether there is difference at DNA level between the curly and wild type stocks.

METHODS AND MATERIALS

The study began on May, 2006, ending on March, 2008.

Fly stocks: Curly stocks used in the study includes Cy antimorphic allele, 67.3, In(2LR)En/Cy[1] from Bloomington Drosophila stock center at Indiana University (BDSC), Df(2L)N19/Cyo from Drosophila Genetic Resource Center (DGRC) at Kyoto Institute of Technology and the wild-type, Oregon from National Institute of biological sciences at Beijing, China.

DNA extraction: Total genomic DNA was extracted from fresh flies from the curly stocks and the wild type with the procedure described by Sullivan *et al.* (2000) and then stored at -20° for latter PCR amplifications.

Design of primers: The primers used for PCR amplification were designed according to the sequence (264961.....271261) from www.ncbi.nlm.nih.gov/genbank (Accession AE003582.4). It contains the whole genomic interval and parts of genes Syt and daw.

RESULTS

Among 14 pair primers used to amplify the genomic interval, a restricted fragment length Polymorphism was detected between the Cy stocks and the wild type when PCR was conducted with the eighth pair primers. There was one band in the electrophoresis pattern of PCR products amplified from the wild type while two bands appeared in those of the Cy stocks in which one band was identical to the band of the wild type(A band) and another one had a smaller size (B band) (Fig. 1).

Given that the curly is heterozygous character, genomic DNA extracted from wild and curly F1 flies from crosses of three curly stocks and Oregon, was amplified with the eighth pair primers. In the curly F1 flies the PCR products also contained the same two bands (A and B band) as those in curly parents, but only A band in the wild F1 flies.

This result indicated that the B fragment is closely associated with Cy mutant chromosomes. Further, Sequencing analysis revealed that A band contains a fragment of 234 bp which is identical to a sequence from Genbank (Accession AE003582.4) while B band is a fragment of 132bp which has a 102 bp deletion compared with A band (Fig. 2A). In addition, all fragments amplified with other 13 pair primers were analysed by sequencing and blast, showing that no DNA difference was found between the Cy stocks and the wild type.

In the further study using the deletion as a marker was to determine genotypes of flies from Cy67.3 and the wild type, Oregon during different developmental stages: embryo, the first star

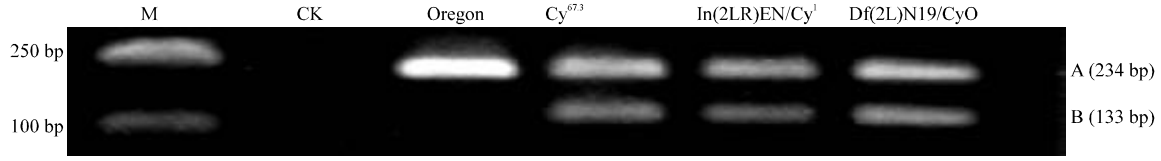

Fig. 1: PCR products amplified from Curly and wild-type flies. Forward 5'TTAAGTGGCCCCTTATTC 3';Reverse 5'CTAAGCACCCATCGAAAT 3'. The PCR reaction was implemented under the following conditions: denaturing at 94° for 4 min, 30 cycles of denaturing at 94° for 30 sec, annealing at 62° for 30 sec and extension at 72° for 20 sec, followed by at 72° for 5 min. The PCR products were electrophoresed on 1.2% agarose gel and stained with EBr .There is one band in the electrophoresis pattern of PCR products amplified from wild while two bands appear in that of Curly in which one band is identical to the band of wild (Fig. 1A)and another has a smaller size (Fig. 1B). Given that Curly is a dominant heterozygous character, genomic DNA extracted from individual flies from Curly F1 from Curly and wild was also amplified , the PCR product also contains the same two bands as those of their parents

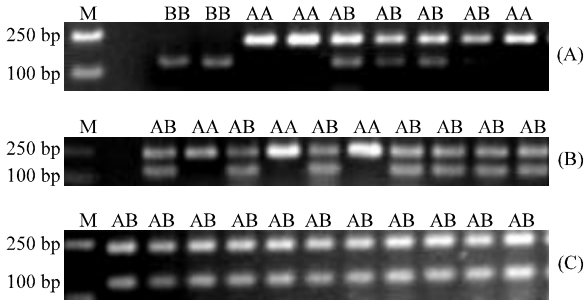

Fig. 2: Detecting genotypes of flies during different developmental stages in Cy67.3. Forward 5'TTAAGTGGCCCCTTATTC 3';Reverse 5'CTAAGCACCCATCGAAAT 3'. The PCR reaction was implemented under the following conditions:denaturing at 94° for 4 min, 30 cycles of denaturing at 94° for 30 sec, annealing at 62° for 30 sec and extension at 72° for 20 sec, followed by at 72° for 5 min. The PCR products were electrophoresed on 1.2% agarose gel and stained with EBr . In Cy67.3, (A) flies with BB genotype were found only on embryo period., (B) flies with AA being detected on the first star larva, the second star larva, the third star larva until pup and (C) flies with AB genotype showing on all different developmental

larva, the second star larva, the third star larva and pupa. In the wild type only flies with AA genotype were found on all different developmental stages, but in Curly strains flies with BB genotype were found only on embryo period, flies with AA being detected on the first star larva, the second star larva, the third star larva until pupa while flies with AB genotype showing on all different developmental stages (Fig. 3). It showed that in Curly strain Flies with homozygote of Cy chromosome (BB genotype) and its homology (AA genotype) have been dead before adult.

Whether are there unusual genomic features in the region in which the deletion occurs? A prediction of transcription factor binding sites was completed on http://www.genomatix.de/cgi-

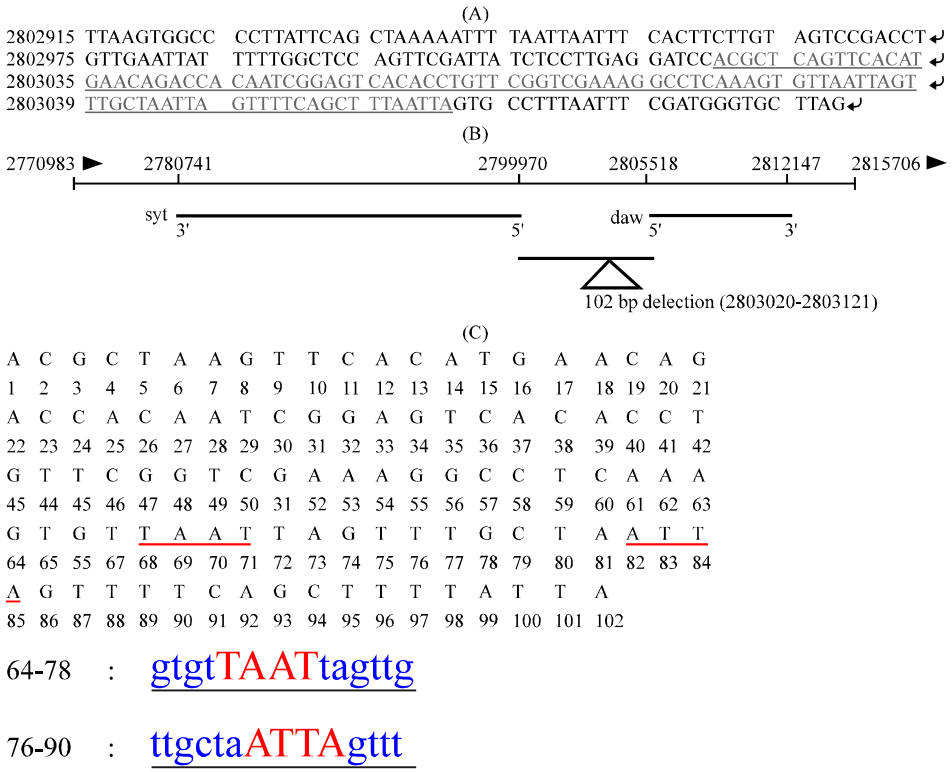

Fig. 3: (A) Comparison of sequence between A band and B band. The PCR products were purified using the PCR product purification kit (SANGON, shanghai), then cloned into PMD vector 18T (Takara, Japan). Cloned PCR products were sequenced using the ABI BigDye3.1 terminator Cycle sequencing Ready Reaction kit, ABI 3730XL sequencer. A Band-a fragment being equal to a sequence from Genbank under accession No. AE003582.4 (268231-268464), the equivalent of 2802915-2803139 on genome; B Band-a fragment which has a 102 bp deletion compared with A Band. Continous line representing a 102 bp deletion part, identical to a sequence from Genbank under accession AE003582.4 (268336-268437), the equivalent of 2803020-2803122 on genome; (B) schematic mapping the region in which the deletion occurs on; (C) The prediction of transcription factor bind sites for the region in which the deletion occurs. The prediction was completed on http://www.genomatix.de/cgi-bin /matinspector_pro. 64-78 and 76-90 representing two predicted transcription factor binding sites. TAAT or ATTA is core sequence of transcription binding sites of homeoproteins

bin/matinspector_pro. The prediction showed that two transcription binding sites which contain TAAT or ATTA, core recognition sequences of homeoproteins in drosophila melanogaster (Dessain *et al*., 1992; Ekker *et al*., 1992, 1994; Gehring *et al*., 1994; Biggin and McGinnin, 1997), are located in the region in which the deletion occurs (Fig. 3C). Homeoproteins have been major determinants on developmental processes in multicellular organisms from drosophila to mammalians. Moreover, DNA sequences of their recognition sites plays a important role in biological function of homeoproteins (Ekker *et al*., 1992, 1994; Gehring *et al*., 1994; Biggin and McGinnin, 1997).

DISCUSSION

A Cy chromosome that carries no visible cytological rearrangement was isolated previously (Lindsley and Zimm, 1992). Littleton and Bellen (1994) reported that four Cy chromosomes displayed no obvious rearrangement, one containing an inversion with a breakpoint at 23B1-23B2 and one carrying a more complex rearrangement with a breakpoint at 23B1-23B2 (Littleton and Bellen, 1994). Here, first we report a 102 bp deletion at DNA level which is located on the genomic interval between Genes syt and daw, corresponding to 23B1-23B2 on chromosome. The deletion invariably appears in different Curly strains including Cy antimorphic allele, 67.3 (Littleton and Bellen, 1994), In(2LR)En/Cy^1, Df(2L)N19/Cyo and two curly stocks from our lab, another one from Beijing University in previous study (Li *et al*., 2006). These Cy mutant chromosomes are not derived from the same parental chromosome, showing that the deletion arose independently.

Is it possible the deletion we found somehow effects a promoter/enhancer in daw protein, resulting in abnormal expression or lack there of in the wing discs?in further study more proofs will be required for investigating if deletion of the transcription binding sites is responsible for curly phenotype. In a word, the 102 bp deletion is interesting for better understanding the molecular mechanism underlying the Curly phenotype.

CONCLUSION

Firstly a 102 bp deletion which locates the genomic interval between genes syt and daw has been found to be associated with the Cy mutant chromosome. Moreover, it is interesting for better understanding the molecular mechanism underlying curly phenotype.

ACKNOWLEDGMENTS

We are grateful to Li-hua Bai for making medium of drosophila in the study, Doctor Xue mei Deng for some helpful suggestions on molecular methods, Bloomington Drosophila stock center at Indiana University (BDSC) for providing Cy67.3, In (2LR)En/Cy^1, National Institute of biological sciences at Beijing, China for Oregon stocks and Drosophila Genetic Resource Center of Kyoto Institute of Technology for Df(2L)N19/Cyo.

REFERENCES

Adams, M.D., S.E. Celniker, R.A. Holt, C.A. Evans and J.D. Gocayne *et al*., 2000. The genome sequence of *Drosophila melanogaster*. Science, 287: 2185-2195.

Ashburner, M., C. Detwiler, S. Tsubota and R.C. Woodruff, 1983. The genetics of a small autosomal region of *Drosophila melanogaster*, including the structural gene for alcohol dehydrogenase. VI. Induced revertants of scutoid. Genetics, 104: 405-431.

Bennett, K., N. Calakos and R.H. Scheller, 1992. Syntaxin-a synaptic protein implicated in docking of synaptic vesicles at pre-synaptic active zones. Science, 257: 255-259.

Biggin, M.D. and W. McGinnis, 1997. Regulation of segmentation and segmental identity by *Drosophila* homeoproteins: The role of DNA binding in functional activity and specificity. Development, 124: 4425-4433.

Dessain, S., C.T. Gross, M.A. Kuziora and W. McGinnis, 1992. Antp-type homeodomains have distinct DNA binding specificities that correlate with their different regulatory functions in embryos. EMBO J., 11: 991-1002.

Ekker, S.C., D.P. von Kessler and P.A. Beachy, 1992. Differential DNA sequence recognition is a determinant of specificity in homeotic gene action. EMBO J., 11: 4059-4072.

Ekker, S.C., D.G. Jackson, D.P. von Kessler, B.I. Sun, K.E. Young and P.A. Beachy, 1994. The degree of variation in DNA sequence recognition among four *Drosophila* homeotic proteins. EMBO J., 13: 3551-3560.

Gehring, W.J., Y.Q. Qian, M. Billeter, K. Furukubo-Tokunaga and A.F. Schier *et al.*, 1994. Homeodomain-DNA recognition. Cell, 78: 211-223.

Leveque, C., T. Hoshino, P. David, Y. Shoji-Kasai and K. Leys *et al.*, 1992. The synaptic vesicle protein synaptotagmin associates with calcium channels and is a putative Lambert-Eaton myasthenic syndrome antigen. Proc. Nat. Acad. Sci. USA., 89: 3625-3629.

Li, H.W., L.S. Lian, C.J. Zhao, L.H. Bai, X.M. Deng and C.X. Wu, 2006. Identification of a common deletion in different curly mutants in *Drosophila melanogaster*. Yi Chuan, 28: 1254-1258.

Lindsley, D.L. and G.G. Zimm, 1992. The Genome of *Drosophila melanogaster*. Academic Press, San Diego, USA.

Littleton, J.T., M. Stern, K. Schulze, M. Perin and HJ. Bellen, 1993. Mutational analysis of *Drosophila synaptotagmin* demonstrates its essential role in Ca++ activated neurotransmitter release. Cell, 74: 1125-1134.

Littleton, J.T. and H.J. Bellen, 1994. Genetic and phenotypic analysis of thirteen essential genes in cytological interval 22F1-2: 23B1-2 reveals novel genes required for neural development in *Drosophila*. Genetics, 138: 111-123.

Parker, L., J.E. Ellis, M.Q. Nguyen and K. Arora, 2006. The divergent TGF-ligand *Dawdle* utilizes an activin pathway to influence axon guidance in *Drosophila*. Development, 133: 4981-4991.

Petrenkao, G., M.S. Perin, A. Davletov, A. Ushkaryov and M. Geppert *et al.*, 1991. Binding of synaptotagmin to the a-latrotoxin receptor implicates both in synaptic vesicle exocytosis. Nature, 353: 65-68.

Serpe, M. and M.B. O'Connor, 2006. The metalloprotease tolloid-related and its TGF like substrate *Dawdle* regulate *Drosophila* motoneuron axon guidance. Development, 133: 4969-4979.

Spencer, F.A, F.M. Hoffmann and W.M Gelbart, 1982. Decapentaplegic: A gene complex affecting morphogenesis in *Drosophila melanogaster*. Cell, 28: 451-461.

Sullivan, W., M. Ashburner and S. Hawley, 2000. *Drosophila* Protocols. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, ISBN: 0879695862.

Ward, L., 1923. The genetics of curly wing in drosophila. Another case of balanced lethal factors. Genetics, 8: 276-300.