Molecular characterization of a point mutation in the *Drosophila inscuteable* gene.

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Asymmetric cell divisions play a crucial role in establishing different cell fates in various tissues, reviewed *e.g.* in Chia *et al.* (1997), Knoblich (1997), Lin and Schagat (1997), Jan and Jan (1998), Lu *et al.* (1998), Paululat *et al.* (1999). During embryonic development the specification of muscle progenitors and neuroblasts depends on the asymmetric distribution of intrinsic cell factors (Ruiz Gomez and Bate, 1997; Carmena *et al.*, 1998). Several proteins involved in this process were identified in *Drosophila*, including Inscuteable (Kraut and Campos-Ortega, 1996; Knirr *et al.*, 1997), Numb (Uemura *et al.*, 1989; Rhyu *et al.*, 1994; Knoblich *et al.*, 1995), Prospero (Hirata, *et al.*, 1995; Knoblich, Jan *et al.*, 1995; Spana and Doe, 1995), Miranda (Ikeshima-Kataoka *et al.*, 1997; Shen *et al.*, 1997), Sanpodo (Dye *et al.*, 1998; Park *et al.*, 1998; Skeath and Doe 1998), Bazooka (Kuchinke *et al.*, 1998) and Staufen (Li *et al.*, 1997; Shen *et al.*, 1998). Inscuteable (Insc) is one of the key components responsible for the asymmetric localization and distribution of *e.g.* Numb and contains a number of conserved motifs, some of which were functionally analyzed recently (Tio *et al.*, 1999). *Insc* mutants show severe defects in cell fate determination of muscle progenitors and neuroblasts (Burchard *et al.*, 1995; Kraut and Campos-Ortega, 1996; Kraut *et al.*, 1996; Knirr, Breuer *et al.*, 1997).

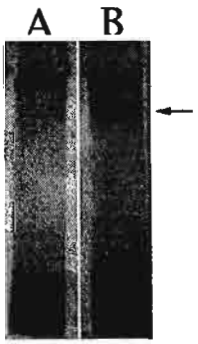

Figure 1. SSCP analysis. PCR amplification products from heterozygous *insc*^{22} flies (A) show conformational changes (arrow) when compared to a control (B, isogenic strain, except for the *insc* locus).

Here we show the molecular analysis of the EMS-induced *insc*^{22} allele (previously described as *nem*^{22}; (Burchard, Paululat *et al.*, 1995) that reveals a C to T transition resulting in a truncated protein of 82 aa, respectively.

For the sequence analysis we isolated genomic DNA from an embryonic collection that was enriched for *insc*^{22} homozygous embryos (heterozygous individuals hatch as larvae and were removed prior to DNA preparation). Based on the known *insc* sequence, primer pairs were designed that allowed the amplification of overlapping fragments of 200 to 300 base pairs in length, encompassing the entire coding region, the second and the third intron (Knirr, Breuer *et al.*, 1997). The first intron of about 10 kb in length and the untranslated regions of *insc* were not tested. PCR amplification products were applied to 10% polyacrylamide gels and used for a single strand conformation analysis (SSCP) (Savov *et al.*, 1992; Hongyo *et al.*, 1993).

A primer pair used for the amplification of 247 bp including the 3'end of the second exon and the 5' end of the second intron generates a fragment that reveals conformational polymorphism in comparison to a wildtype control (Figure 1).

For the sequence analysis, a PCR reaction was performed with the same primers as mentioned above using enriched *insc*^{22} DNA as a template. The amplification products were subcloned into the pPCR-Script vector (Stratagene) and six independent clones were sequenced. Two clones turned out to be wild type while four clones show an identical point mutation.

The sequence analysis of the DNA from homozygous *insc*^{22} embryos revealed a C to T transition 250 bp downstream of the ATG codon of the *insc* cDNA as a result of the EMS mutagenesis (Figure 2A, B).

The CAG in the wild type, respectively the TAG in *insc*^{22}, is the last codon of the second exon. Exon/intron boundaries of the second *insc* intron show highly conserved sequences that match the consensus splicing sites (Sharp, 1987; Mount *et al.*, 1992; Wassarman and Steitz, 1992). Thus 5'splicing of the second *insc* intron occurs downstream of the CAG (Gln) codon (Figure 3). Therefore in the *insc*^{22} allele the C to T

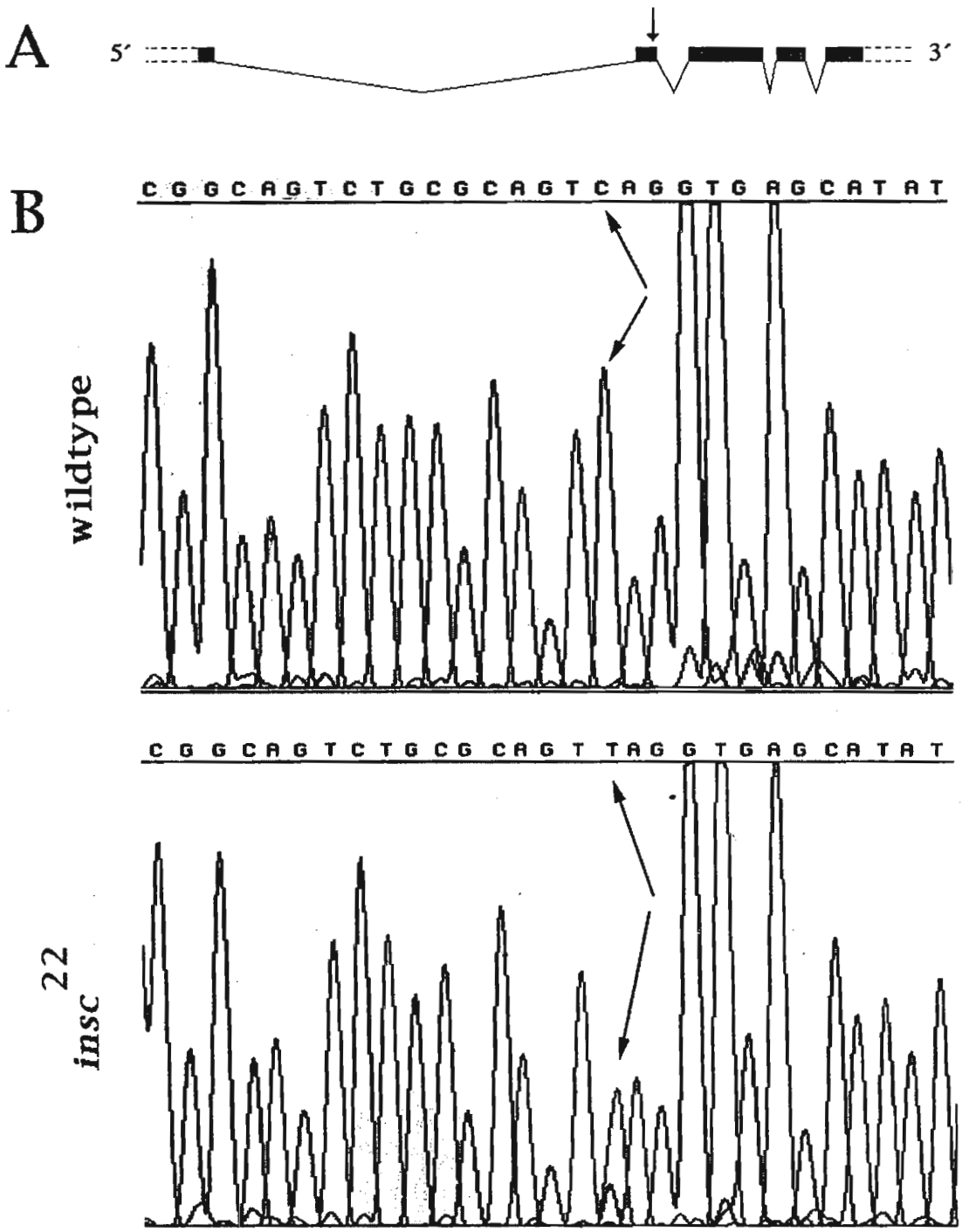

Figure 2. (A) shows the exon/intron structure of the *inscuteable* gene. The point mutation in *insc*^{22} is indicated by an arrow. Sequence analysis (B) of *insc*^{22}-DNA reveals a C to T transition (arrows) when compared to wildtype DNA.

transition in this codon likely creates an artificial stop codon resulting in a truncated Insc protein of 82 amino acids instead of 860 amino acids in the wild type.

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wildtype sequence

CTG	CGC	AGT	CAG	GTGAG -//- TTTG CAG	GAT	TCG
Leu	Arg	Ser	Gln	intron (247 bp)	Asp	Ser

insc^{22} sequence

CTG	CGC	AGT	TAG	GTGAG -//- TTTG CAG	GAT	TCG
Leu	Arg	Ser	**Stop**	intron (247 bp)	Asp	Ser

Figure 3. The second *insc* intron (boxed) shows highly conserved splice site sequences (see text for references). The C to T transition in *insc*^{22} occurred in the last CAG codon of the second exon, coding for Gln.

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