Xstir Polymorphism and Absence of Sex Linkage in *Xenopus laevis ME2* Gene

(*Xenopus laevis* / malic enzyme 2 / polymorphism / Xstir / intron)

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Abstract. A fragment of ME2 cDNA from exon 2 to exon 11 was sequenced and the sequence submitted to GenBank. Analysis of the intron, probably intron 13, revealed a polymorphism which is due to the presence of tandem repetitions of Xstir elements. Genetic analysis of the parents and the offspring showed a standard distribution of intron variants. This distribution was not dependent on sex. We conclude, contrary to previous reports, that the *ME2* gene is not linked to sex. Consequently, the Xstir polymorphism can be used as a tool for genetic analysis.

In developmental biology *Xenopus laevis* is a widely used model organism, yet its genetics is far from clear so far due to its long generation time and few well-characterized genetic markers. Almost all members of the *Xenopus* genus are of polyploidic origin and form a polyploid series of 2n, 4n, 8n, 12n starting with diploid *X. tropicalis* and tetraploid *X. laevis* (Tymowska and Kobel, 1972; Tymowska and Fishberg, 1973; Kobel and Du Pasquier, 1986). During meiosis *X. laevis* forms bivalents (Tymowska and Fishberg, 1973). This reveals its ancient, tetraploid, strongly diploidized character, which is consistent with the high proportion of gene duplications in its genome (review Graf and Kobel, 1991). Breeding sex-reversed males with normal males produces only male progeny (Chang and Witschi, 1955, 1956; Gallien, 1955, 1956). Crosses of sex-reversed males and sex-reversed females result in all female progeny (Mikamo and Witschi, 1964). These results indicate the Abraxas type sex determination in *X. laevis*, in other words: the *Xenopus* male is homogametic (ZZ) and the *Xenopus* female is heterogametic (WZ).

The genetics of *X. laevis* was studied mostly by electrophoretic analysis of isoenzymes (Graf, 1989b). The same method was used for determination of the sex linkage of mitochondrial malic enzyme (Graf, 1989a). Here the author detected malic enzyme activity in the mitochondrial extract from liver of frogs bred by backcrossing hybrids of four *X. laevis* subspecies with respective parental subspecies. The enzymes were separated by starch gel electrophoresis at pH 6.0 and detected histochemically. Based on the results of the segregation analysis, the author concluded that alleles do segregate as expected for a sex-linked gene with 6% of recombinants. Malic enzyme (ME) catalyzes the oxidative decarboxylation of malate to pyruvate. It is found in three forms coded by separate genes. Cytoplasmic malic enzyme is labelled as ME1, mitochondrial ME2 and ME3. ME2 and ME3 differ in structure and isoelectric point. ME3 is the basic enzyme with isoelectric point of approximately 9.0. These properties exclude ME3 as the protein detected in Graf's study (1989a). During electrophoresis at pH 6.0, ME3 migrates catodically and cannot be detected under such circumstances.

Heterochromosomes of *X. laevis* were not identified despite a detailed morphological analysis (Schmid and Steilein, 1991). Therefore, we searched for a sex-linked marker gene appropriate for chromosomal localization. Since the malic gene showed the lowest recombination with sex, we were interested in its further characterization, with the prospect of using it as a heterochromosome marker.

Material and Methods

RNA and DNA were isolated from *X. laevis* liver by the guanidine hydrochloride method (Kingston and Gilman, 1994-1997) and the proteinase K – phenol method (Moore, 1994-1997). RT-PCR and PCR were performed according to manufacturer's instructions (MBI Fermentas, Vilnius, Lithuania). Cloning was done using the TOPO TA cloning kit (Invitrogene, San Diego, CA).

The human ME2 exon-intron structure, deduced from comparing the genomic contig NT_033905 (GenBank) and messenger RNA sequence NM_002396 (GenBank), was used to establish the provisional exon-intron structure in *X. laevis*. Primers, fitted to the exon-intron structure of human ME2, were designed using *X. laevis* EST sequences similar to the human *ME2* gene (GenBank

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Abbreviations: MDH – malate dehydrogenase, ME – malic enzyme.

BG730283, BE508129, BG406935). Primers XM2F2 (5'-GCA AGA AAG GCA AAT CCT CGA – 3') and XM2R4 (5' – CGT CTT CTG TAA ACA GCC GTC C – 3') expected to span from exon 2 to exon 11 were used for RT-PCR. Primers XM2F7 (5' – AGT TCG ACA CAT AAG TGA CCG GTT – 3') and XM2R6 (5' – CCT GGT CCC ATC TCT TCA ACA G – 3') expected to span from exon 13 to exon 14 and containing the sequence of the intron were used for analysis of intronic polymorphism. Sequenation was done by AGOWA (Berlin, Germany).

Results and Discussion

RT-PCR yielded a partial *X. laevis* ME2 sequence that was submitted to GenBank (AY225508). The corresponding protein sequence was compared with human ME2 (Fig. 1), showing 80% identity. The comparison of the *X. laevis* and human ME2 protein sequences confirmed that the chosen EST corresponds to the *ME2* gene, since these sequences are in substantial length overlapping with the RT-PCR sequence. Since the primers used for genomic analysis are complementary to sites within this EST sequence, we can assume that the observed polymorphism occurs in the *ME2* gene of *X. laevis*.

The analysis of one couple and its offspring using PCR with primers XM2F7 and XM2R6 demonstrated polymorphism in the respective *ME2* gene segment (Fig. 2). The fragments were labelled as follows: 670 bp as A, 940 bp as B and 1100 bp as C. The male parent had B and C fragments and the female parent A and C fragments. Offspring (42 males, 26 females) had an AB:AC:BC:C distribution of 19:12:14:23. This result is in agreement with the distribution of alleles a/c and b/c with 17a/b:17a/c:17b/c:17c/c ratio ($\chi^2 = 4.35 < \chi^2_{0.05} = 7.8$). All offspring combinations were of both sexes (a/b 58% males, a/c 50%, b/c 50%, c 78% males). In the case of the complete sex linkage only two alelic combinations can occur in each sex in the offspring, which is far from observed results. Theoretical frequences without sex linkage (10.5 males and 6.5 females for each allelic combination) are not significantly different from experimental values (11:6:7:18 in males and 8:6:7:5 in females) according to the χ^2 test ($\chi^2 = 9.2 < \chi^2_{0.05} = 14.1$).

These results are in contrast to the results of Graf (1989a). There are two different explanations for the discrepancy. The first rests on the possibility that there is another *ME2* gene linked to sex. The second possibility is that Graf worked with mitochondrial malate dehydrogenase MDH2. Both enzymes react with NADP and NAD and produce the histochemical detection pattern used by Graf.

Sequencing of the fragment A containing an intron, probably intron 13 (Fig. 3), revealed the presence of tandem repetitions of 86 bp elements corresponding to

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ME2 X.laevis LQGLLPPKIESQDIQAARFHRNLSRIDDPLQKYIYLMGIQERNEKLFYRVLLDDIEHLMP
ME2 Homo     ..........T.....L......KKMTS..E....I.............I.Q....S...

ME2 X.laevis IVYTPTVGLACSQYGHIFRRPKGLYISILDRGHIPSILDHWPETDVKAVVVTDGERILGL
ME2 Homo     ........................F...S....VR..V.N...NH..............

ME2 X.laevis GDLGVYGMGIPVGKLCLYTACAGIRPQTCLPVLIDVGTDNPSLLKDPFYMGLYQKRDRTQ
ME2 Homo     ...........................DR....C.......IA..................

ME2 X.laevis LYDELIDEFMDAVTDRYGQNTLIQFEDFGNHNAFRFLRKYREKYCTFNDDIQGTASVALA
ME2 Homo     Q..D......K.I.....R.....................................A....

ME2 X.laevis GMLAAQKAIRKPITEHRILFLGAGEAALGIANLIVMSMMEHGISAEAARERIWMFDQFGL
ME2 Homo     .L.....V.S...S..K.....................V.N.L.EQE.QKK.....KY..

ME2 X.laevis LIQGRGEGIDGNQELFAHSAPEKPVSSFLDAVKVLQPTAIIGVSGA
ME2 Homo     .VK..KAK..SY..P.T.....SIPDT.E...NI.K.ST....A..
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Fig. 1. Sequence comparison of *X. laevis* ME2 sequence (GenBank AY225508) and human ME2 sequence (GenBank NM_002396).

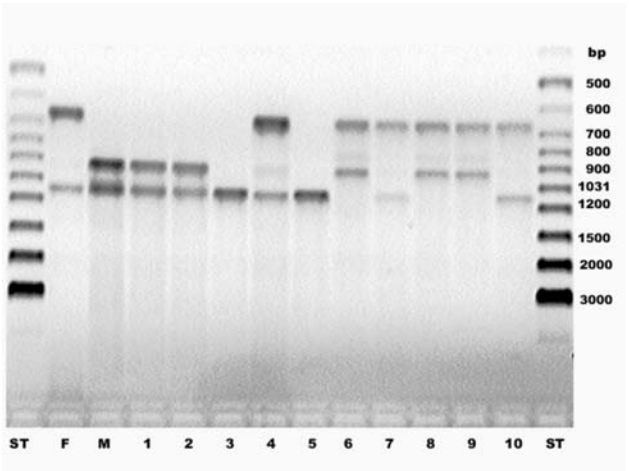

Fig. 2. Distribution of intron variants in parents and offspring (ST = standard DNA fragments, F = female, M = male, 1–10 = offspring number).

TTTTTCTGAAGCTGCTAAGgtcagttgcttaacctgcaatatttatttaataaaaagcatc
acatgtgacgtgtaggcatgtctccatgtatgactcttgataatacagtagataacagac
aagtactactatagtttatataaacaagctgtgtagccatgggggcagacattcaagcac
aggatacacagtagataacagataagtactactatagtttatataaacaagctgctgtgt
agccatgggggcagccattcaagcacaggatacacagtagataacagataagtactacta
tagtttaaataaacaagctgctgtgtagccatgggggcagccattcaagcacatgatata
caccctctcctattcatattccagacttttattcaaatcaatgcatggttgttaggagaa
tttggaccatagcaaccaaattgctcaaattgcaaactggagagctgctgaataaaaagc
taaataataaaaattgaaaaccaactgcaaatgttctcagaatatccctctctacatcat
tgcaaaagttaattgaaaggtgaacagcctctttaaacatgaaatgtgtattttaataaa
tgtagGCGCTTGCTGAGCAATTGA

Fig. 3. Sequence of fragment A (GenBank AY 225509) containing an intron sequence with three Xstir repetitions. Homology with the Xstir sequence (GenBank AB039922) shown in grey (non-homologous bases within the Xstir sequence shown in white). Upper case marks exonic sequences, bold face represents the beginning of each repetition and underlined are *Sca* 1 cleavage sites.

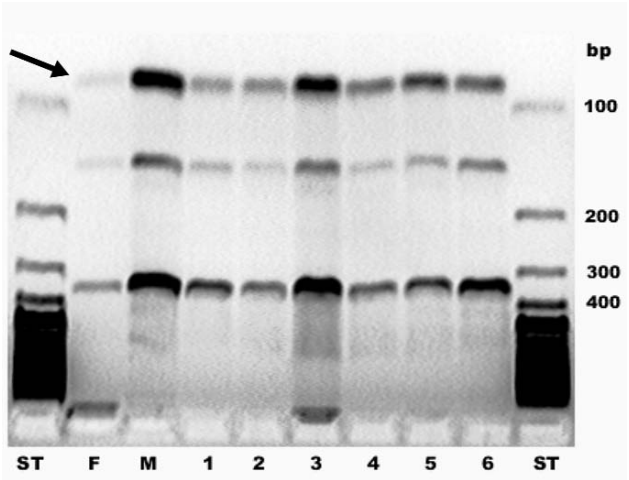

Fig. 4. Sca 1 DNA restriction of intron fragments (F = female, M = male, 1–6 = offspring number corresponding to Fig. 2, arrow = 86 bp Xstir band).

Xstrir as described by Hikosaka et al. (2000). The repetitions contain a *Sca* 1 restriction site. The restriction of PCR products from parents and offspring containing fragments A, B and C and their combinations with *Sca* 1 resulted in a uniform pattern of 86 bp and flanking sequence bands (Fig. 4). This demonstrates the presence of 3, 6 and 8 Xstir tandem repetitions. This is the first described example of Xstir polymorphism. We have evidence of a broad distribution of this marker (unpublished results). Therefore, Xstir repetitions could become a useful marker for segregation analysis.

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