

IV. CONCLUSIONS

1. We have sequenced *X. laevis* ME2 gene and discovered Xstir length polymorphism in its intron 13 with the standard Mendelian inheritance. Found polymorphisms in ME2 gene could be used as a marker for linkage or physical map construction.
2. We have developed an efficient method for localization of single-copy genes on *Xenopus laevis* chromosomes on the basis of fluorescence *in situ* hybridization coupled with tyramide amplification step (FISH-TSA).
3. As the first group we were able to localize in the *Amphibia* class a single-copy gene, *c-src1*, to the subcentromeric region of two homologous *X. laevis* chromosomes belonging to category G.
4. Mdh2 paralogous genes of *X. laevis* have been sequenced and their exon-intron structure has been determined.
5. The FISH-TSA method is able to differentiate between genes with 95% similarity. We succeeded in localizing and efficiently distinguishing two paralogous Mdh2 genes to the subcentromeric region of long arms of two chromosomes 3 (Mdh2a) and two chromosomes 8 (Mdh2b). Found intronic polymorphisms present in both genes provided standard ratios according to Mendelian inheritance.
6. Linkage to sex of Mdh2 paralogous genes has not been confirmed as well as ME2 gene. Graf's linkage data (1989a, 1989b) were thus disproved.
7. We sequenced and localized Mdh2 gene of *Xenopus tropicalis*. The FISH-TSA method originally developed for *X. laevis* reveals Mdh2 gene in the subcentromeric region of long arms of chromosome 3 just as with *X. laevis*.
8. Genes with known structure, chromosomal position, and easily detectable alleles are suitable for ordering contigs and joining base pair maps with linkage analysis. High intron mutation rate is also of general use for linkage analysis. The FISH-TSA method employs short cDNA sequences for chromosomal localization and can facilitate a genome analysis at least in *Vertebrata*.