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Body color mutants and a transposable element affecting the tyrosinase gene of the medaka fish, *Oryzias latipes*

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Abstract In the medaka fish *Oryzias latipes*, many mutants for body colors have been isolated. Among them, albinos (*i*-locus alleles) are typical color mutants having amelanotic skin and red -colored eyes with no tyrosinase activity. To cast light on the molecular basis of the albino mechanism, we have cloned cDNAs for tyrosinase, a key enzyme in melanin biosynthesis, from the wild-type fish, and performed Southern blot analysis on genomic DNA from an *i* mutant fish with the cDNA probe. The results demonstrated a 1.9-kb insertion fragment in exon 1 of the tyrosinase gene. The fragment, designated *Tol*, (i) carries terminal inverted repeats containing sequences quite similar to those of the *Tc1* transposable element, (ii) is flanked by duplicated segments of their host chromosomes, and (iii) is present in approximately 80 copies in the genome, showing specific features of the *Tc1*-like transposable element. It is clear that insertion of *Tol* into the tyrosinase gene causes the albino body color in the *i* mutant.

As a contrast to albino-*i* alleles, there is a colorless melanophore mutant *b*, having normally pigmented black eyes but orange-colored skin with amelanotic melanophores, thus showing the presence of a tissue-specific mechanism of melanin formation. Using the cDNA probe, Northern blot analysis on poly(A)$^+$RNAs from various tissues was carried out. In the wild-type, the tyrosinase mRNA was expressed in eyes and skin but not in liver, corresponding to tissue-specific melanin formation. In the *b* mutant, contrary to expectation, the mRNA was detected not only in eyes but also in amelanotic skin. Therefore, pigmentation of skin controlled by *b* is not directly related to expression of the tyrosinase gene.

Introduction

Medaka fish, *Oryzias latipes*, is a useful animal for studying vertebrate genetics and embryology. One of the best explored areas is the genetics of color variants and more than 40 mutants affecting body colors have been isolated and maintained (Tomita, 1975). Among them, spontaneous albino mutants (*i*-locus alleles) are typical color mutants having amelanotic skin and red-colored eyes with no tyrosinase activity (Tomita, 1975). In addition to the albino-*i* allele cases, there is a colorless melanophore mutant which shows orange body color but eyes with normal pigmentation, thus evidencing tissue-specific formation of melanin granules. This trait is controlled by *b*-locus alleles with the mutant being homozygous for recessive *b* (Aida, 1921, 1930), whose biochemical basis is not known.

Tyrosinase (EC 1.14.18.1) is a key enzyme in melanin biosynthesis, catalyzing the oxidation of tyrosine to dopa and then dopa to dopaquinone (Körner and Pawelek, 1982). This enzyme is found in various types of pigmented cells, including melanophores in skin and pigmented epithelial cells in eyes. We therefore considered that a nucleic acid probe for the tyrosinase gene might be an invaluable tool for studies of the regulation of tyrosinase expression in various medaka fish color mutants. In the present study, we isolated and sequenced tyrosinase cDNAs and used them as a probe to analyze not only the genomic structure of the gene in the *i* mutant but also expression of the gene in the *b* mutant.

Materials and Methods

(a) Fish strains

The following mutants of *O. latipes* were obtained from Dr. Tomita, Laboratory of Freshwater Fish Stocks at Nagoya University: a spontaneous albino mutant *BiR* (phenotype albino-*i*, genotype *i/i* for the *i* locus), a colorless melanophore mutant *bIR* (phenotype *b*, genotype *b/b*) and its wild-type *BIR* (brown skin color; wild phenotype for well -characterized three skin color loci, *b*, *i* and *r*). The wild-type of skin color is commonly designated in this way.

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(b) DNA and RNA extraction

High molecular weight DNA was extracted from bodies of fish through proteinase K treatment, phenol extraction and ethanol precipitation by standard techniques (Sambrook *et al.*, 1989). A single fish was used for each variant. Total RNAs were extracted from eyes, skin and liver of about 500 individual *BIR* and *bIR* fish by the acid-guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). Poly(A)$^+$RNAs were isolated from total RNAs as described previously (Inagaki *et al.*, 1994).

(c) Probe synthesis by the reverse transcriptase (RT)-PCR reaction

Comparing the known tyrosinase cDNA sequences of the mouse (Yamamoto *et al.*, 1987; Müller *et al.*, 1988), human (Kwon *et al.*, 1987), chicken (Mochii *et al.*, 1993) and frog (Takase *et al.*, 1992), a sense primer A (5'-AAGGAATG-TTGTCCAGTCTGG-3') and an antisense primer B (5'-GCATCTCTCCAGTCCCAGTATGG-3') were designed and synthesized on a DNA synthesizer (Pharmacia-LKB, Upsala). Using these primers, a probe designated *TyrF* was synthesized from poly(A)$^+$RNA from eyes of the *BIR* fish with the reverse transcriptase (RT)-PCR reaction, and labeled with [α-^{32}P] dCTP as reported previously (Inagaki *et al.*, 1994).

(d) cDNA cloning and DNA sequencing

A lambda-gt10 cDNA library was constructed from 2 μg samples of the poly(A)$^+$RNA from the *BIR* fish with oligo-dT primer (Sambrook *et al.*, 1989) using cDNA synthesis and cDNA cloning system kits (Amersham). We screened this library using the *TyrF* DNA probe described above. Positive signals were subcloned into pBluescript II KS+ (Stratagene, La Jolla). Generating nested sets of deletions, representative cDNA clones were sequenced on both strands by the dideoxy chain termination method (Sanger *et al.*, 1977) using BcaBest Dideoxy Sequencing Kit (TaKaRa, Kyoto).

(e) Analysis of sequence data

The DNASIS program (Hitachi Software Engineering, Tokyo) was used for sequence data compilation and to search sequences for reading frames, transcriptional control signals, and inverted or direct repeats, as well as to obtain a hydropathy profile of the amino acid sequence and to do a data base search.

(f) Southern blot analysis

High molecular weight DNAs were digested with several restriction enzymes, fractionated on 0.8% agarose gels, and transferred to nylon membranes (Hybond-N; Amersham) followed by UV irradiation. The membranes were then hybridized with a ^{32}P-labeled probe by the standard technique (Sambrook *et al.*, 1989). The probe *TyrG* was a genomic *Bgl*II fragment of the *BIR* fish containing *TyrF*, being labeled with [α-^{32}P] dCTP to about 1×10^9 cpm/μg using Multiprime DNA labeling system (Amersham). Hybridization conditions were 6× SSPE (20× SSPE ; 3 M NaCl, 0.2 M NaH_2PO_4, 0.02 M EDTA, pH 7.4), 0.5% SDS, 5× Denhardt's reagent and 50 μg/ml of fragmented salmon sperm DNA at 65°C for 16 hrs.

(g) Northern blot analysis

10 μg samples of poly(A)$^+$RNA from liver, eyes and skin of the *BIR* fish, and eyes and skin of the *bIR* fish were electrophoresed on 1.2% (w/v) formaldehyde denaturing agarose gels, transferred to nylon membranes (Hybond-N, Amersham), immobilized by UV irradiation, and then hybridized with ^{32}P-labeled cDNA probes under the conditions described previously (Inagaki *et al.*, 1994). As an internal standard, a probe for β-actin gene was generated by (RT)-PCR from liver RNA of the *BIR* fish using actin-specific primers.

Results

(a) Cloning and sequencing of tyrosinase cDNA

Using *TyrF* as a probe, we screened the cDNA library and isolated two types of clones, a major clone *bcTyr1* and a minor longer clone *bcTyr8*. Clone *bcTyr1* is 2.2 kb in size, and clone *bcTyr8* has an additional 1 kb fragment at its 3' site. The nucleotide (nt) sequence of *bcTyr1* was completely determined and published elsewhere (Inagaki *et al.*, 1994). Fig. 1 shows the sequence of *bcTyr8* in which the main part of *bcTyr1* is included. The additional region of *bcTyr8* has no ORF and contains a highly AT-rich sequence. Thus, the longer clone may be generated by alternative processing.

(b) Predicted amino acid sequence of medaka fish tyrosinase

Fig. 1 also shows the predicted amino acid sequence. The cDNA codes for 540 amino acids beginning at nt 322 to nt 1,944. Using a package program (Kyte and Doolittle, 1992) with DNASIS, the hydropathy profile of the amino acid sequence

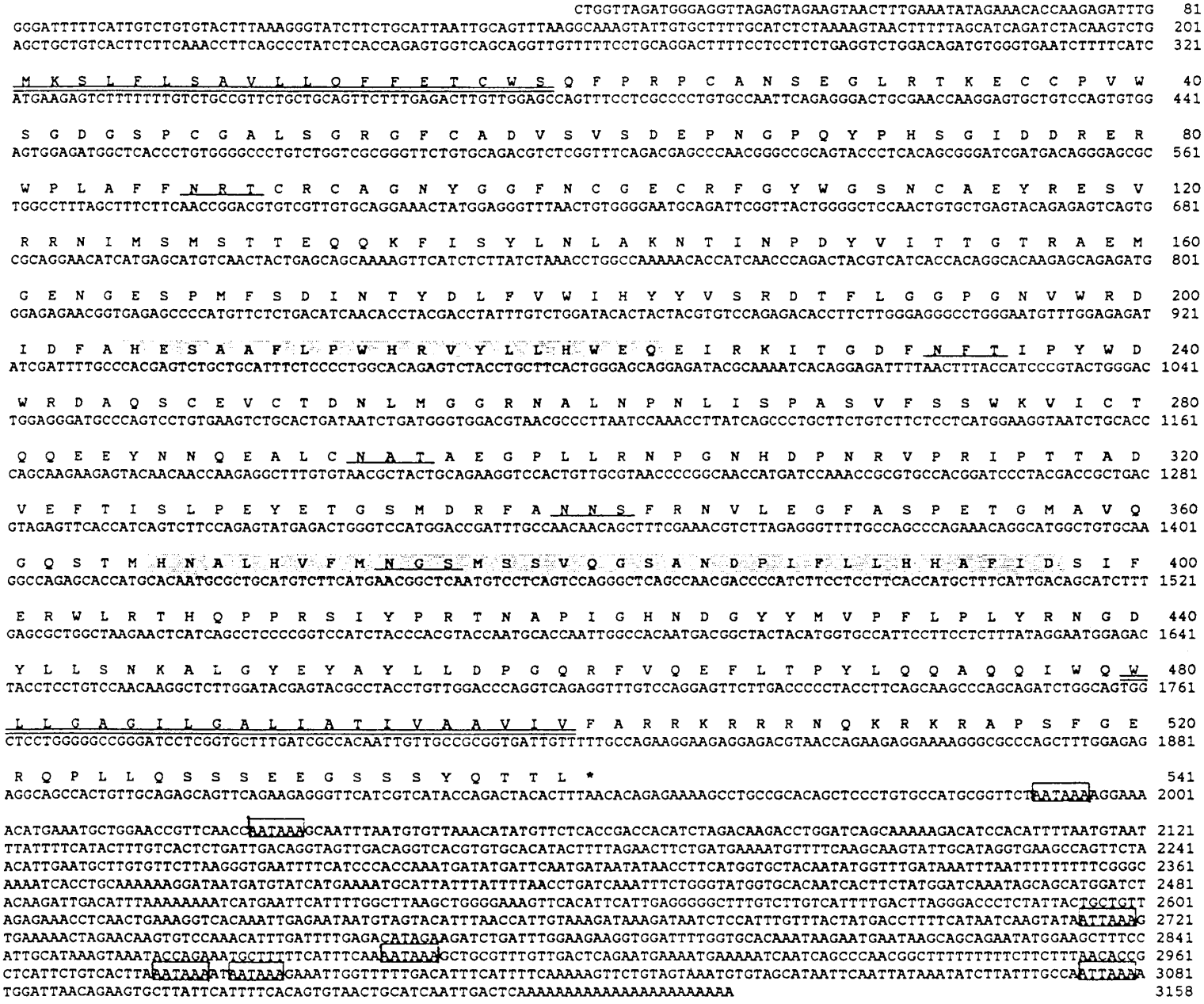

Fig. 1. Nucleotide sequence of the medaka fish tyrosinase cDNA *bcTyr8* and the deduced amino acid sequence. Numbering of the nucleotide sequence corresponds to that of *bcTyr1* as previously described (Inagaki *et al.*, 1994). The *bcTyr8* specific sequence is from nt 2049 to nt 3158. The six polyadenylation signals (AATAAA or ATTAAA) in the 3' untranslated region are boxed. The derived ORF beginning at nt 322 of the initiation codon to the stop codon at nt 1942 is depicted in one letter code. High hydrophobic regions (double underlined) at the N-terminal and near the C-terminal are in the signal peptide and transmembrane regions, respectively. Putative copper binding sites are indicated by shaded boxes. The five potential N-glycosylation signals are underlined.

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Human       ML.AVLYC..WS.Q.SAGH...A.VS.KN.ME.....P....R....Q.....S.QNILL.NA.  64
Mouse       MF.AVLYC..WS.QISDGH...A..S.KN.LA.....P.M.......Q.....S.Q.ILL.SA.  64
Chicken     MF..AMGL..VILQPSTG....V...TQS.LR.....P.D...T...ER.N..T.QRILL.QA.  64
Frog     MESTTV.LATST..LVLHASYG....A.STAQV.LS.......P..N.S..EV....V.Q..VP.NS.  68
Medaka      MKSLFLSAVLLQFFETCWSQFPRPCANSEGLRTKECCPVWSGDGSPCGALSGRGFCADVSVSDEP  65
                                          *          **         *         *

Human    L...F.FT.V....S..SV.Y....Q.S..FM.....N.K..F..P..T.R.LL.....FDL.AP.KD 132
Mouse    S...F.FK.V....S..SV.Y....Q.S..FM.....N.K..FG.P..T.K.VLI....FDL.VS.KN 132
Chicken  L...F.F..V....D..SV.Y.....R..FM.....K..FS.Q..T.R.LRT....FQLTIS.KD 132
Frog     V.A.F.F.......N..IV.Y....Q.Q..FM.Y.........T.P..TVR.NMI.KD.FR.T.A.KD 136
Medaka   NGPQYPHSGIDDRERWPLAFFNRTCRCAGNYGGFNCGECRFGYWGSNCAEYRESVRRNIMSMSTTEQQ 133
                                  * *         * *       *

Human    ..FA..T...H..SS....PI..YGQ.-K..ST...N...I......M.....M.AL...-SEI.... 198
Mouse    ..F...T...H..SSV...P...YGQ.-N..ST...N...I......M........L...-SEI.... 198
Chicken  ..LA.......IPSK....A...Y.Q.-N..SN...RN..V......M...A....L...-S...... 198
Frog     .L.A......H..S.....A...YEQ.-N..SN...A..SA..........A...A..ED-.S..A.. 202
Medaka   KFISYLNLAKNTINPDYVITTGTRAEMGENGESPMFSDINTYDLFVWIHYYVSRDTFLGGPGNVWRDI 201

Human    .....AP.......LF..R.....Q.L...E............EK.DI...EY...QHPT....L... 266
Mouse    .....APG......LF..L.......EL...E...V........EN.DI...EYL...HPE....L... 266
Chicken  .....APG......AF..L..R..Q.....E............ED.VI...EY...QHPT....L... 266
Frog     .....APG......F...L..R..Q.V...D.....F.......Q..L...EFF..THPTSN..L... 270
Medaka   DFAHESAAFLPWHRVYLLHWEQEIRKITGDFNFTIPYWDWRDAQSCEVCTDNLMGGRNALNPNLISPA 269
            I                                                 * *

Human    .F....QIV.SRL....SHQS...G.P....R.......KSTT..L.SS.....CL..TQ..S....K 334
Mouse    .F....QI..SRS....SHQV..DG.P............KAKT..L.SS.....CL..TQ..S..... 334
Chicken  .F....Q.....S....S.Q......S...I......N.KS.T..L.SSSE...CLT.TQ..S....K 334
Frog     .F....QI..SRP....SLRII..G.N......S..R..R..T..L..S....ACL..TD....A... 338
Medaka   SVFSSWKVICTQQEEYNNQEALCNATAEGPLLRNPGNHDPNRVPRIPTTADVEFTISLPEYETGSMDR 337
                 *             *

Human    A..F....T.......L..I.DAS..S......IY...T..Q...............V.....Q..Q 402
Mouse    T..F....T..V....L..I.DPS..S......I....T..Q...............V.....Q... 402
Chicken  M..Y....T.....D.H.AISNIS..GL.....IY......Q..........I.....V..... 402
Frog     S..F....T.....V.TS.I.NRS..S...S....L...............V.....V..L..Q... 406
Medaka   FANNSFRNVLEGFASPETGMAVQGQSTMHNALHVFMNGSMSSVQGSANDPIFLLHHAFIDSIFERWLR 405
                                          II

Human    R.R.LQEV..EA.......RES.....I.......FFI.S.D...D.S..Q.SDPDSF.DYIKS..E. 470
Mouse    R.R.LLEV..EA.......RDS.....I.......FFITS.D...D.S..QESDPG.YRNYIE...E. 470
Chicken  R.R.MLEV..AA.......REN.....I......EFFI.SRE...D.E..QE.ALGSF.D..I...K. 470
Frog     R...SLDV..EA...V...RE.N....I..FT..EFFVQSRD...D.D..AES--GSIED..L...E. 472
Medaka   THQPPRSIYPRTNAPIGHNDGYYMVPFLPLYRNGDYLLSNKALGYEYAYLLDPGQRFVQEFLTPYLQQ 473

Human    .SR..S.....AMV..VLTALL.GPVSLLC--.HKRK-----QLPE.K....MEKEDYH.L-..SH.  529
Mouse    .SR..P.....ALV..V..AALSGLSSRLCLQKKKKK.----QPQE......MDKDDYH.LL..SH.  533
Chicken  .H...P..V..AVI.GI.TAVLSGL.-L.C--.KKRK-----GTSP.I....TE.EDYNNV...SHF  529
Frog     .R.........AV..G..TAVI.TI.SLTC--..KRKTK----ISE.TR...MEAEDYQ-AT..SN.  532
Medaka   AQQIWQWLLGAGILGALIATIVAAVIVFARRKRRRNQKRKRAPSFGERQPLLQSSSEEGSSSYQTTL 540
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Fig. 2. Alignment of the amino acid sequences of vertebrate tyrosinases. Five N-glycosylation sites (NXS and NXT) are underlined. Conserved cysteine residues were indicated by asterisk. I and II represent highly conserved regions of putative copper binding sites in tyrosinases, tyrosinase-related proteins and hemocyanin molecules. Shadows indicate the conserved cysteines, aromatic residues and glycines in a characteristically spaced pattern seen in an EGF-motif (Jackson *et al.*, 1992).

was analyzed. High hydrophobicity was found at the N-terminus and near the C-terminus (data not shown). According to von Heijne's method (von Heijne, 1983), 19 amino acids at the N-terminus are predicted to constitute a signal peptide found in secreted proteins. The C-terminal hydrophobic region is a putative membrane spanning domain. Five potential N-glycosylation sites (Asn-X-Ser/Thr) are present in the sequence (Fig. 1).

Alignment of the tyrosinase amino acid sequences from vertebrate species (Fig. 2) indicates that the medaka fish tyrosinase shows about 60% identity to those of other vertebrates, *i.e.*, human, mouse, chicken and frog. This high homology indicates that the isolated clones are indeed from the tyrosinase gene. Five potential N-glycosylation signals and fourteen cysteine residues (Fig. 2) are shared with other tyrosinase sequences, indicating their potential importance for tyrosinase function and conformation. Conservation of cysteines, aromatic residues, and glycines in a characteristically spaced pattern is well known as an EGF-like motif which is important for protein-protein recognition (Jackson *et al.*, 1992). The motif found at amino acid positions 90 to 113 was also found conserved in the alignment (Fig. 2). Müller *et al.* (1988) identified two histidine sites in homologous regions of tyrosinases, tyrosinase related proteins and hemocyanins serving as the copper-binding sites, the active center of tyrosinase. This postulation is consistent with our observation that histidines in these regions are completely conserved.

*(c) Structural change of the tyrosinase gene in the albino-*i *medaka fish*

In order to characterize the albino mechanism of the *i* mutant, Southern blot analysis of *BIR, bIR* and *BiR* genomic DNAs was carried out with several restriction enzymes using the *TyrG* probe. Fig. 3 is an autoradiograph for *Hin*dIII, demonstrating strong signals for single genes in all DNAs, indicating that the tyrosinase gene is present as a single copy (Inagaki *et al.*, 1994). Note here that *BiR* always shows a signal length larger by approximately 1.9 kb than *BIR* and *bIR*. Several DNA blot analyses using other restriction enzymes also gave the same result, indicating that the albino-*i* fish carries an extra DNA fragment in or near the probed region (Fig. 3). The extra fragment is inside the first exon of the tyrosinase gene and its genomic structure is shown schematically in Fig. 4.

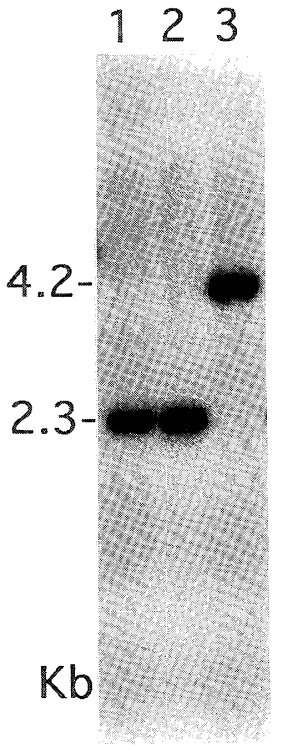

Fig. 3. Southern blot analysis of tyrosinase genes. 10 μg samples of genomic DNA from *O. latipes BIR* (wild-type; lane 1), *bIR* (orange color variant; lane 2) and *BiR* (albino-*i* mutant; lane 3), digested with *Hin*dIII and hybridized to the probe *TyrG*.

Using a clone containing the extra 1.9 kb fragment as a probe, Southern blot analysis of *BIR, BiR* and *bIR* was carried out. The results indicated the presence of multicopies of the fragment in all DNAs (data not shown), suggesting that the fragments are repetitive. The copy number of the elements in the genome of *O. latipes* is estimated to be roughly 80. We have designated these repetitive sequences as *To1*. The particular copy found in the tyrosinase gene is denoted *To1-tyr*.

(d) Properties of transposable elements

The whole *To1-tyr* element and its flanking regions were completely sequenced (Koga *et al.*, 1994), its length being 1854 bp. A characteristic feature is that the outside ends of *To1-tyr* have clear terminal inverted repeats, flanked by an 8 bp apparent target site duplication. The repeats consist of 14 nucleotides with one mismatch, having characteristic sequences of CAGTAG at one end and CAGTGG at the other, quite similar to the consensus sequence CAGT(A/G)(T/C) of the *Tc1* DNA-mediated transposable element family (Rozenzweig *et al.*, 1983a, 1983b; Harris *et al.*, 1988). Though the nucleotide sequence of *To1-tyr* was compared with other sequences in

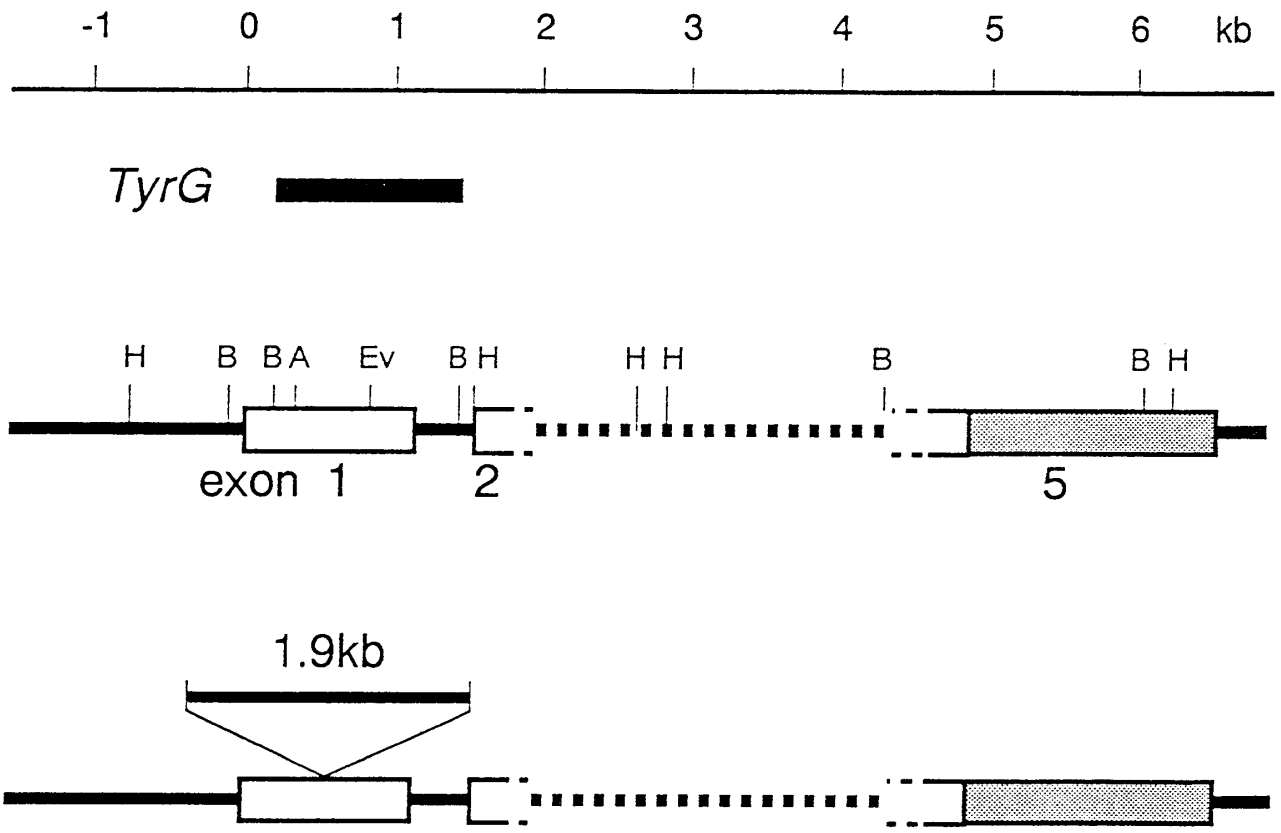

Fig. 4. Schematic representation of the tyrosinase gene of the albino-*i* mutant. The upper diagram maps the positions of restriction sites with the locus of the wild-type tyrosinase gene. The lower diagram shows the same locus of the albino-*i* mutant, containing a *To1* element. The lengths of fragments expected in *Hin*dIII and *Apa*I digests are shown for both versions. The tyrosinase-coding regions common to *bcTyr1* and *bcTyr8* and specific for *bcTyr8* are indicated by the open and shaded boxes, respectively. Regions from exon 2 to 5 that have not been completely determined are represented by dashed boxes and lines. *TyrG*: a *BIR* fish genomic *Bgl*II fragment containing part of exon 1 of the tyrosinase gene. Restriction sites are abbreviated as follows; A, *Apa*I; B, *Bgl*II; Ev, *Eco*RV; H, *Hin*dIII. Not all sites are shown.

GenBank and EMBL data banks, with special attention to sequences of known eukaryotic transposable elements, no similar sequence was found. Furthermore, no plausible coding frames were detected in *To1-tyr*. These findings suggest that this particular copy is not likely to be an autonomous member.

(e) Tissue-specific expression of the tyrosinase gene in wild-type medaka fish

To know whether tyrosinase mRNA is preferentially expressed in various pigmented tissues, Northern blot analysis was performed with the cDNA clone *bcTyr1* as a probe (Fig. 5A). In the wild-type *BIR* fish, a signal of 2.2 kb corresponding to the tyrosinase mRNA appeared in eyes and skin (Fig. 5A, lanes 2, 3), but was not detected in liver (Fig. 5A, lane 1). The transcript was most abundant in eye tissue, and the results confirm tissue specific expression of the tyrosinase gene in the medaka fish. Thus, the production of tyrosinase may be mainly regulated at the transcriptional level as in mice (Ruppert *et al.*, 1988), human (Kwon *et al.*, 1987) and chickens (Mochii *et al.*, 1993).

(f) Alternative processing in tyrosinase gene expression

On Northern blot analysis with *bcTyr1*, we could detect minor signals larger in size (4.3 kb and 5.6 kb) in addition to the strong signal of 2.2 kb (Fig. 5A). To characterize these signals, the same membrane was rehybridized to the 3'-additional sequence specific to *bcTyr8*. The probe could hybridize only the larger fragments, and the major signal of 2.2 kb in Fig. 5A disappeared (Fig. 5B). Therefore, these transcripts larger in size seem to contain the specific sequence for *bcTyr8*. Since the tyrosinase gene is a single copy in the genome, the additional sequence of *bcTyr8* adjoins the 3' region of the abundant clones. This indicates that the larger clone *bcTyr8* is generated by

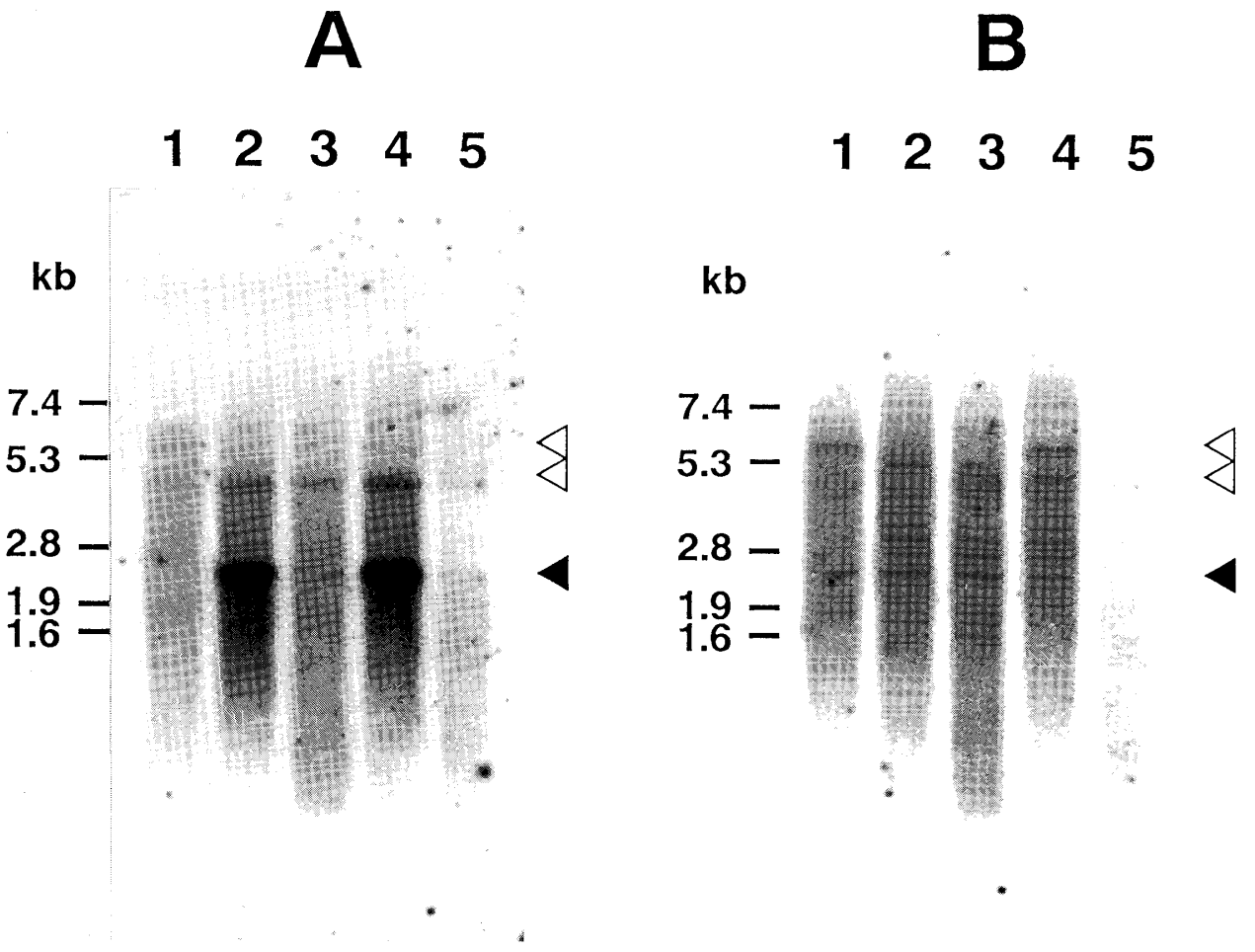

Fig. 5. Northern blot analysis of tyrosinase gene transcripts in different tissues and different types of medaka fish. **A.** Expression of the tyrosinase gene compared between *BIR* (lanes 1-3) and *bIR* (lanes 4, 5). RNAs were extracted from liver (lane 1), eyes (lanes 2, 4), and skin (lanes 3, 5) from both fish. 10 μg samples of poly(A)$^+$RNAs were loaded in each lane. The cDNA clone *bcTyr1* was used as a probe for the tyrosinase mRNA. Closed and open arrowheads indicate the major 2.2 kb signal and the larger transcripts, respectively. The same membrane was rehybridized to the β-actin probe as an internal control (data not shown). **B.** The same membrane was rehybridized to the probe for the additional sequence specific for *bcTyr8*. Open arrowheads, larger transcripts.

alternative processing (Inagaki *et al.*, 1994). However, the significance of the multiple transcripts for the common function of vertebrate tyrosinases is not clear (Müller *et al.*, 1988). Note here that a small but detectable amount of the larger transcripts but no detectable hybridization signal for the normal transcript were apparent in liver RNA (Fig. 5A, lane 1), suggesting an unknown function of tyrosinase in the liver.

(g) Tyrosinase gene expression in the colorless melanophore mutant

In the wild-type *BIR* fish, melanization is detected in the eyes, skin and peritoneum. On the other hand, the colorless melanophore mutant *bIR* has no pigmented melanophores in the skin in contrast to the normal pigmentation in their eyes and peritoneum (Tomita, 1975). To know whether tyrosinase mRNA is lacking in skin, Northern blot analysis of *bIR* was performed with the probe *bcTyr1* as described above. Contrary to expectation, we could detect the same signals not only in the eyes but also in the skin of *bIR* (Fig. 5A, lanes 4, 5). Although the skin of *bIR* is not pigmented, the tyrosinase gene is thus clearly expressed in melanophores at the mRNA level. We therefore conclude that the *b* allele does not suppress transcription of the tyrosinase gene.

Discussion

*(a) albino-*i *mutant*

Originally, *i* was a spontaneous mutant isolated from a breeding population of medaka fish. The recessive allele-*i* causes the albino body color in

its homozygous carriers that show no tyrosinase activity *in vivo* (Tomita, 1975). A link between the *i* locus and the tyrosinase gene has been proposed because defective tyrosinase genes result in the albino phenotype in the mouse (Beermann *et al.*, 1990; Yokoyama *et al.*, 1990; Tanaka *et al.*, 1990) and human (Takeda *et al.*, 1990). In the present study, we could show that the albino-*i* carries a 1.9 kb extra DNA fragment *Tol-tyr* in its tyrosinase gene. It is likely that the expression or translation of the tyrosinase mRNA must be interrupted by the element and this provides strong evidence of a causal correspondence.

(b) Tissue-specific expression of the tyrosinase gene in the b *mutant*

When Hishida *et al.* (1961) incubated scales of the *b* mutant (*bIR*) in the presence of iodoacetamide and tyrosine, a number of artificially melanized cells appeared, showing the presence of the tyrosinase activity. In this reaction, tyrosine is a substrate for tyrosinase, and iodoacetamide acts as a blocker of sulfhydryl bonding. Based on their histochemical data, they claimed that the colorless melanophores of *bIR* must contain tyrosinases in an inactive state and some treatments such as iodoacetamide seem to remove the "inhibitor" of tyrosinase. Recently, however, using transgenic medaka fish, Matsumoto *et al.* (1992) demonstrated that *bIR* can present brownish skin pigmentation as a result of introduction of the mouse tyrosinase gene into the genome. Contrary to the "inhibitor" hypothesis described above, the mock gene could be expressed and activated in the colorless melanophore. One possibility is thus that the authentic tyrosinase gene is not expressed in reality and that the tyrosinase activity shown by scales is an artifact.

In the present study, we found that the tyrosinase gene is certainly expressed in skin of *bIR*, as well as in the wild-type. This indicates that tyrosinase mRNA or the tyrosinase enzyme *in vivo* is present in an inhibited state in *b*, which would imply the presence of an "inhibitor(s)" or the absence of an "activator(s)" of melanin synthesis in skin melanophores. Therefore, the *b* locus gene product must regulate melanin formation in skin by some mechanism independent of normal expression of the tyrosinase gene. Why this should not block the action of tyrosinase originating from an introduced mouse gene remains unclear but one plausible explanation might be that the "inhibitor" itself can not interact with the exogenous gene product. Another explanation is that overproduction of enzyme due to the double gene dose effect of both authentic and exogenous genes overcomes the "inhibitor".

(c) A transposable element in medaka fish

We have described a transposable element in medaka fish termed *Tol*, especially *Tol-tyr*, whose conspicuous feature is inverted repeats at its termini, flanked by a duplication of a segment of the host chromosome. Furthermore, partial sequences of the inverted repeats are quite similar to those of *Tc1* which was originally described in the nematode species *Caenorhabditis elegans* (Rosenzweig *et al.*, 1983a). These repeats and duplicated host sequences are the hallmark of DNA-mediated transposable elements (Howe and Berg, 1989). Furthermore, Southern blot analysis of the genomic DNA clearly demonstrated the presence of multicopies of the elements. The dispersed locations of *Tol* further suggest a transposable capacity. It is therefore likely that *Tol* is a member of the *Tc1*-like DNA-mediated transposon family.

Because complete versions of virtually all known DNA-mediated transposable elements encode a product necessary for their own movement, the autonomous transposable elements are characterized by short inverted terminal repeats flanking putative transposase-encoding reading frames (see Howe and Berg, 1989). Since *Tol-tyr* has no convincing ORFs in its sequence, it is not likely to be an autonomous member, although other versions of *Tol* might contain such genes.

While several distinct families of DNA-mediated transposable elements are known in prokaryotes, lower eukaryotes, plants and invertebrates (see Howe and Berg, 1989), only a few candidates have so far been identified in vertebrate genomes. Hosts are limited to frog species of *Xenopus* (Kay and David, 1983; Schubiger *et al.*, 1985; Garrett and Carrol, 1986; Gerber-Huber *et al.*, 1987) and a cyclostome (Heierhorst *et al.*, 1992). Among them, cyclostome *Tes1* is the only *Tc1*-like element in vertebrates known to date. In the present paper, we showed that *Tol* must be a member of the *Tc1* family in the genome of the vertebrate *O. latipes*. This indicates a much wider phylogenetic distribution of the *Tc1* family than previously thought. It remains to be established whether this broad distribution is due to evolutionary conservation or to horizontal transfer of these elements across species borders, which has been

proposed for other transposon families. We expect that further data will allow the molecular evolution of the *Tc1*-family to be discussed in the near future.

Tc1 elements are the major cause of spontaneous mutations in certain *C. elegans* strains, and have successfully been used as an agent for screening and cloning of several genes (Moerman and Waterston, 1989). Unfortunately, no evidence for transposase activity was obtained for *To1*, and it is thus premature to comment on the mutational impact of *To1* elements in the medaka fish genome.

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