Mutations affecting xanthophore pigmentation in the zebrafish, *Danio rerio*

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SUMMARY

In a large-scale screen for mutants with defects in embryonic development we identified 17 genes (65 mutants) specifically required for the development of xanthophores. We provide evidence that these genes are required for three different aspects of xanthophore development. (1) Pigment cell formation and migration (*pfeffer* and *salz*); (2) pigment synthesis (*edison*, *yobo*, *yocca* and *brie*); and (3) pigment translocation (*esrom*, *tilsit* and *tofu*). The number of xanthophore cells that appear in the body is reduced in embryos with mutations in the two genes, *salz* and *pfeffer*. In heterozygous and homozygous *salz* and *pfeffer* adults, the melanophore stripes are interrupted, indicating that xanthophore cells have an important function in adult melanophore pattern formation. Most other genes affect only larval pigmentation. In embryos mutant for *edison*, *yobo*, *yocca* and *brie*, differences in pteridine synthesis can be observed under UV light and by thin-layer chromatography. Homozygous mutant females of *yobo* show a recessive maternal effect. Embryonic development is slowed down and embryos display head and tail truncations. Xanthophores in larvae mutant in the three genes *esrom*, *tilsit* and *tofu* appear less spread out. In addition, these mutants display a defect in retinotectal axon pathfinding. These mutations may affect xanthophore pigment distribution within the cells or xanthophore cell shape. Mutations in seven genes affecting xanthophore pigmentation remain unclassified.

Key words: pigment cells, chromatophores, xanthophores, pigment pattern, pigment translocation, pteridines, maternal effect

INTRODUCTION

Skin coloration in fish and amphibia is created by the interaction of light with pigmented cells (melanophores, xanthophores and iridophores), that are derived from the neural crest (DuShane, 1934; Rawles, 1944). Within these cells, pigments are located in specialized pigment organelles such as melanosomes, which contain melanin (Charles and Ingram, 1959; Drochmans, 1960; Birbeck, 1963); pterinosomes, which contain pteridines in xanthophores (Matsumoto, 1965a; Kamei-Takeuchi and Hama, 1971; Bagnara, 1976); and the reflecting platelets which contain purines in iridophores (Bagnara and Stackhouse 1961; Bagnara et al., 1979). Pterinosomes, like melanosomes, are derived from the Golgi complex (Obika, 1993). They contain a species-specific set of pteridines that appear simultaneously with the differentiation of xanthophores (Matsumoto et al., 1960; Hama, 1963; Obika, 1963; Matsumoto, 1965b). Sepiapterins, drosopoterins and several colourless pteridines can be detected as yellow pigmentation that first becomes visible within pterinosomes (Obika, 1963; Kamei-Takeuchi and Hama, 1971). These pteridines are largely the same as the pteridines described for *Drosophila* eye pigmentation (Matsumoto, 1965a; Bagnara, 1966; Matsumoto and Obika, 1968; Frost and Malacinski, 1980).

Pigment organelles in individual chromatophores can be transported along uncharacterized paths radiating from the cell center, so that the organelles are either dispersed throughout the cytoplasm or aggregated in the center of the cell (Taylor, 1992). The distribution of pigment organelles within pigment cells is important in determining the general colouration of the body. Cells with dispersed organelles give rise to the general colour pattern of the animal, whereas chromatophores with aggregated organelles appear less coloured (reviewed by Luby-Phelps and Schliwa, 1982; Obika, 1986).

Several mutations have been described in different amphibian species that affect different aspects of vertebrate pigmentation (Frost and Malacinski, 1980; Frost et al., 1982; Droin, 1992). To gain a more detailed insight into the different aspects of vertebrate pigmentation it would be helpful to study a collection of mutations in one species. The zebrafish is an ideal system for using the powerful tools of genetics to study different aspects of vertebrate pigmentation (Mullins et al., 1994; Mullins and Nüsslein-Volhard, 1993; Haffter et al.,...
Mutations affecting the development of the adult pigment pattern in the zebrafish have been described previously (Kirschbaum, 1975; Johnson et al., 1995). Zebrafish xanthophore pigmentation first becomes apparent after 42 hours of development (high pec stage) in the dorsal head as a very pale cast of yellow (Kimmel et al., 1995). However, individual xanthophore cells are difficult to distinguish. After 3 days of development (protruding mouth stage), strong yellow xanthophore pigmentation is apparent in the entire dorsal half of the body in regions devoid of melanophores. Xanthophores become granular after 5 days of development and are now seen throughout the body, with greater numbers dorsally. Adult zebrafish have five alternating blue-black (melanophores and iridophores) and silvery-yellow (xanthophores and iridophores) stripes aligned parallel to the long axis of the body. This stripe pattern extends into the caudal and anal fins, but not into the dorsal and the paired lateral fins (Kirschbaum, 1975). Studies of adult pigmentation mutants revealed interactions between melanophores and iridophores in pigment pattern formation (Johnson et al., 1995). In contrast, little is known about the interaction of the xanthophores with other chromatophores (Epperlein and Löbberg, 1984; Goodrich et al., 1994).

In a screen for mutants with defects in embryonic development, many embryonic pigmentation mutants were isolated that allow the study of different aspects of pigment cell development (Kelsh et al., 1996). In this paper we describe mutations affecting the development of xanthophore pigmentation. We provide evidence that genes involved in three different aspects of xanthophore development were isolated in the screen. Mutations in salz and pfeffer lead to an adult pigment pattern phenotype that is probably due to a reduced number of xanthophore cells in the body. Mutations in edison, yobo, yocca and brie affect the pteridine content and so probably function in pigment synthesis or uptake. Mutations in esrom, tilsit and tofu affect retinotectal axon pathfinding as well as xanthophore pigmentation, and might be required for pigment translocation or for the generation of normal xanthophore cell shape.

### MATERIALS AND METHODS

**Fish raising, screen and crosses**

Fish stocks were maintained as previously described by Mullins et al. (1996). A total of 284 mutations affecting larval pigmentation were isolated (Kelsh et al., 1996). Of these, 124 mutants (41 genes) show xanthophore pigmentation defects (Table 1 and Kelsh et al., 1996). 20 mutants, defining 14 complementation groups, have defects in all three types of pigment cells, that is the melanophores, the iridophores and the xanthophores. Xanthophores and melanophores are affected in 19 mutants, which make up four complementation groups. Xanthophore and iridophore pigmentation is affected by mutations in at least six genes (20 mutants). In this paper we focus on mutations in 17 genes (64 mutations), which lead to defects in xanthophores without an effect on larval melanophore or iridophore pigmentation. Xanthophore specification or migration are probably affected in salz (sal) and pfeffer (pfe) (Class II, missing cell type in Kelsh et al., 1996), whereas the other genes are probably required for xanthophore differentiation. Mutations affecting xanthophore pigmentation lead to pale

### RESULTS

**Genes required for xanthophore development**

In a large-scale screen for mutations leading to defects during the first 6 days of development, we screened for pigmentation phenotypes on the 3rd and 6th day of development (Haffter et al., 1996). A total of 284 mutations affecting larval pigmentation were isolated (Kelsh et al., 1996). Of these, 124 mutants (41 genes) show xanthophore pigmentation defects (Table 1 and Kelsh et al., 1996). 20 mutants, defining 14 complementation groups, have defects in all three types of pigment cells, that is the melanophores, the iridophores and the xanthophores. Xanthophores and melanophores are affected in 19 mutants, which make up four complementation groups. Xanthophore and iridophore pigmentation is affected by mutations in at least six genes (20 mutants). In this paper we focus on mutations in 17 genes (64 mutations), which lead to defects in xanthophores without an effect on larval melanophore or iridophore pigmentation. Xanthophore specification or migration are probably affected in salz (sal) and pfeffer (pfe) (Class II, missing cell type in Kelsh et al., 1996), whereas the other genes are probably required for xanthophore differentiation. Mutations affecting xanthophore pigmentation lead to pale

<table>
<thead>
<tr>
<th>Affected pigment cell type</th>
<th>Number of mutants</th>
<th>Number of genes (alleles)</th>
<th>Gene names (alleles)</th>
<th>Unresolved (mutants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthophores, Melanophores and Iridophores</td>
<td>21</td>
<td>14 (20)</td>
<td>blanced (1), bleached (3), bleich (1), colourless (2), pech (1), puzzle (1), sahne (1), sallow (1), sunbleached (1), stonewashed (1), u-boot (1), weiss (1), washed out (4), white tail (1)</td>
<td>1 (tc233b)</td>
</tr>
<tr>
<td>Xanthophores and Melanophores</td>
<td>19</td>
<td>4 (17)</td>
<td>cold-light (2), polished (2), stars and stripes (12), tinte (1)</td>
<td>2 (tc249, tz294)</td>
</tr>
<tr>
<td>Xanthophores and Iridophores</td>
<td>20</td>
<td>6 (14)</td>
<td>choco (2), cookie (2), milky (3), marit (1), pistachio (5), vanille (1)</td>
<td>6 (tj266c, tm107, tq262a, tua25b, tz284, tyl105g)</td>
</tr>
<tr>
<td>Xanthophores</td>
<td>64</td>
<td>17 (62)</td>
<td>bressot (1), brie (4), esrom (14), clorix (1), edison (11), feta (1), kefir (2), non blond (1), pfeffer (6), quark(6), ricotta (1), salc (7), tartar(1), tilsit (1), tofu (1), yobo (3), yocca (1)</td>
<td>2 (m14, tua53b)</td>
</tr>
</tbody>
</table>
yellow, whitish or granular cells (Class VI, pale xan-
thophores in Kelsh et al., 1996). Of the 17 genes required for
xanthophore pigmentation, larvae mutant for any of six genes,
brescot (bst), clorix (clx), edison (edi), esrom (esr), non blond
(nob) and tilsit (til), die in larval stages, whereas mutant larvae
for any of the other 11 genes survive to adulthood.

**Xanthophore specification and migration**

In sal and pfe mutant embryos a partial absence of xan-
thophores in the dorsal head becomes apparent after 4 days of
development whereas melanophore and iridophore pigmen-
tation appears normal. 1 day later, large areas devoid of granular
xanthophores can be seen, and only a few individual yellow
spots in the dorsal head are present (arrows in Fig. 1C,E). The
overall number of xanthophores is reduced in pfe larva, as visu-
alized by their fluorescence under UV light (Fig. 1D). In sal
mutant larvae the number of xanthophores is strongly reduced
in lateral and ventral regions, whereas many xanthophores
remain dorsal to the neural tube (Fig. 1F and data not shown).
The strength of the defect in mutant larvae of both genes is
dependent on the allele; weak alleles produce a slightly
reduced number of xanthophores (data not shown), whereas in
embryos with strong alleles only a very small number of xan-
thophores is detectable in the body. For the strong allele
pfe<sup>tg211</sup>, we observed a slight reduction in the number of xan-
thophore cells, and also in heterozygous larvae (data not
shown).

**Heterozygous adult fish**

Heterozygous adult fish carrying a mutation in sal or in pfe
show irregular and interrupted melanophore stripes (shown for
pfe in Fig. 2B), thus indicating a defect in the formation or
maintenance of the adult melanophore pattern. Xanthophores
in the adult heterozygous fish are detectable at a slightly lower
density (data not shown; Haffter, personal communication).
The general appearance is slightly less yellow than wild type
fish. We compared the dominant adult phenotypes to determine
the relative strength of different alleles, which gave the same
results as counting the xanthophore number in the larva
(Table 2).

**Defects in pteridine synthesis in edison, tartar, brie, yocca and yobo**

In edison (edi) larvae no yellow pigmentation is detectable
(Fig. 3C), edi mutant embryos can be detected by the strong
blue fluorescence of embryonic cells including the yolk under

### Table 2. Genes required for xanthophore pigmentation but not for melanophore or iridophore development

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alleles</th>
<th>Adult viability</th>
<th>Other phenotypes</th>
<th>Other descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>pfe</td>
<td>tm236b &gt;tg211 &gt;tg17 &gt;tc227b &gt;tc220 &gt;tg283a</td>
<td>Viable</td>
<td>Dominant and recessive adult phenotype</td>
<td>a,b</td>
</tr>
<tr>
<td>sal</td>
<td>tf241 &gt;tf34 &gt;tp71c &gt;tc254a &gt;tm246b &gt;tb213c &gt;tf238b</td>
<td>Viable</td>
<td>Dominant and recessive adult phenotype</td>
<td>a,b</td>
</tr>
<tr>
<td>edison</td>
<td>tc245c, th232, tl35a, tl245, to255b, tp62, tp67b, tr276, tr232, th04, th253</td>
<td>Lethal</td>
<td>Fluorescence in entire embryo</td>
<td></td>
</tr>
<tr>
<td>tartar</td>
<td>td09</td>
<td>Viable</td>
<td>Fluorescence in entire embryo</td>
<td></td>
</tr>
<tr>
<td>brie</td>
<td>tg211b, tj226a, tn42c, tu269</td>
<td>Viable</td>
<td>Maternal effect</td>
<td>b</td>
</tr>
<tr>
<td>yobo</td>
<td>tc251, tk13, ty44d</td>
<td>Viable</td>
<td>Small iris in adult fish</td>
<td>b</td>
</tr>
<tr>
<td>yocca</td>
<td>tm86</td>
<td>Viable</td>
<td>Retinotectal axon pathfinding</td>
<td>c</td>
</tr>
<tr>
<td>esrom</td>
<td>th241a, te250, te275, tc279, tc376, tf04c, tg05, tg265, th36a, tj236, m207, tp203, ts208</td>
<td>Lethal</td>
<td>Retinotectal axon pathfinding</td>
<td>c</td>
</tr>
<tr>
<td>tilsit</td>
<td>ty130b</td>
<td>Viable</td>
<td>Retinotectal axon pathfinding</td>
<td>c</td>
</tr>
<tr>
<td>tofa</td>
<td>tq121c</td>
<td>Viable</td>
<td>Retinotectal axon pathfinding</td>
<td>c</td>
</tr>
<tr>
<td>bressot</td>
<td>tp222b</td>
<td>Lethal</td>
<td>Retinotectal axon pathfinding</td>
<td>c</td>
</tr>
<tr>
<td>fetu</td>
<td>ty107</td>
<td>Viable</td>
<td>Retinotectal axon pathfinding</td>
<td>c</td>
</tr>
<tr>
<td>kefr</td>
<td>ta65b, tf229 (lost)</td>
<td>Viable</td>
<td>Maternal effect</td>
<td>d</td>
</tr>
<tr>
<td>quark</td>
<td>tc276, tg239d, th236, to241b, tp72g, tv46</td>
<td>Viable</td>
<td>Motility defect</td>
<td>e</td>
</tr>
<tr>
<td>ricotta</td>
<td>th212</td>
<td>Viable</td>
<td>Small otoliths</td>
<td>e</td>
</tr>
<tr>
<td>clorix</td>
<td>tj244</td>
<td>Lethal</td>
<td>General retardation</td>
<td>d</td>
</tr>
<tr>
<td>non blond</td>
<td>tr288</td>
<td>Lethal</td>
<td>General retardation</td>
<td>d</td>
</tr>
</tbody>
</table>

Alleles of different strength are listed according to descending strength separated by >.
References: a, Kelsh et al. (1996); b, Haffter, personal communication; c, Karlstrom et al. (1996); d, Whitfield et al. (1996); e, Granato et al. (1996).
illumination with UV light after 24 hours of development (Fig. 4). This fluorescence subsequently decreases, and by day 6 fluorescence is mainly located in the gut and is excreted. A single allele for tartar (tar) was isolated and this shows a similar but slightly weaker phenotype to that of edi. Weak yellow pigmentation can be detected in tar embryos after 5 days of development (data not shown). Similarly to edi, tar embryos show blue fluorescence in the entire embryo. In mutant edi and tar embryos abnormally shaped and brightly fluorescing xanthophores are present in the body (shown for edi in Fig. 3D). In contrast to edi, homozygous tar fish survive to adulthood and show pale melanophore stripes in the adult (data not shown).

As a first attempt to investigate the synthesis of yellow pigments in the mutants, we performed thin-layer chromatography of larvae to analyze the pteridine components in one allele each of brie, esrom, edison, feta, pfeffer, quark, salz, tartar, yobo and yocca. In wild-type larvae, eight different fluorescent bands can be visualized by UV light on the chromatogram (Table 3). Two bands show visible yellow colour (bands 3 and 7) and therefore are most likely to be sepiapterins (Matsumoto and Obika, 1968; reviewed by Bagnara, 1966). The other six bands, which are colourless under visible light, show fluorescent colours under UV light, are probably colourless pteridines such as biopterin and neopterin (light blue fluorescence), xanthopterin (green fluorescence) and isoxanthopterin (dark blue fluorescence). Correlations between the known pteridines and the eight bands detectable in the wild-type larvae are so far not clear.

Larvae mutant for any of three genes, brie (bri), yocca (yoc) and yobo (yob), show similar pteridine patterns that differ from wild type. The intensity of three fluorescent bands is increased (band 2 increased, band 4 blue instead of green, band 5 strongly increased), and the intensity of two other bands is reduced (bands 6 and 8). For the other seven genes no changes were found.

Xanthophores, as judged by morphological criteria, are different in the three mutants, which show unusual pteridine composition. In bri, cells appear feathery and whitish with normal granularity after 5 days of development (Fig. 3E and data not shown); under fluorescent light these cells appear greenish (Fig. 3F). Xanthophores in yoc mutant larvae appear brownish in the head. Under UV light these cells show a diffuse greenish fluorescence (data not shown). Faintly granular xanthophores with reduced yellow pigmentation are detectable in the head of yob mutant larvae, and fluorescent cells are not detectable in the body (data not shown). Xanthophores in homozygous adult yob, yoc and bri fish appear...
normal. Homozygous \textit{yob} fish do show a seemingly unrelated adult phenotype; the iris in the eye is small (Haffter, personal communication).

**Maternal effect phenotype of \textit{yob}**
Females homozygous for \textit{yob} produce embryos that develop more slowly than embryos derived from heterozygous females or from females homozygous for any of the other viable xanthophore mutations. By day 2, head and tail truncations of variable strengths are observed. The strength of this maternal effect phenotype varies amongst the different alleles. A slow epiboly phenotype was found for embryos derived from a female homozygous for the strongest allele \textit{yob}^{tk13} (data not shown). In embryos derived from a female with the intermediate allele \textit{yob}^{ty44d}, epiboly is normal but the antero-posterior extension is reduced, whereas the axis is broadened laterally (Fig. 5B,D). This leads to a similar but slightly weaker phenotype (Fig. 5F,H) than that produced by the strongest allele described above. In many embryos the eyes are fused in front of the head and the tail is completely missing (Fig. 5F). In the case of the weakest allele, \textit{yob}^{tc251}, some embryos derived from homozygous females are indistinguishable from wild type. The strength of the embryonic phenotype is determined by the mother and is independent of the zygotic genotype (data not shown), indicating that the zygotic function of \textit{yob} is restricted to xanthophore development.

\textit{esr}, \textit{til} and \textit{tof} show defects in xanthophore pigmentation and in the retinotectal axon pathfinding
Overall xanthophore pigmentation is reduced in mutant larvae of \textit{esrom} (\textit{esr}), \textit{tilsit} (\textit{til}) and \textit{tofu} (\textit{tof}) after 5 days of development, although distinct yellow cells are apparent (shown for \textit{til} in Fig. 6C). Xanthophores appear more compact and less granular compared to wild type (arrow in Fig. 6C). Under UV light, the characteristic fluorescent spot at the center of each xanthophore appears brighter and more compact in larvae mutant for each of these three genes than in wild type, whereas the dendritic extensions of the xanthophores appear more spread out in \textit{esr} and \textit{til} larvae (shown for \textit{til} in Fig. 6D).

In addition to the pale xanthophore pigmentation phenotype, mutant \textit{esr}, \textit{til} and \textit{tof} larvae show a defect in retinotectal axon pathfinding (Karlstrom et al., 1996). The occurrence of both phenotypes in \textit{esr}, \textit{til} and \textit{tof} mutants suggests that these genes
may encode components of the cytoskeleton required for both pigment cell morphology and axonal pathfinding of retinal axons.

**Mutants with other xanthophore defects**

Mutations in two genes, *kef1* (*kef*) and *feta* (*fet*), result in changed colours of the xanthophores. In 5-day-old *kef* embryos, whitish cells with faint granularity appear throughout the xanthophore regions, whereas in *fet* mutant embryos brownish cells can be detected in these positions (data not shown). In *quark* (*qua*) mutant embryos the granularity of the xanthophores is strongly reduced, whereas mutations in *ricotta* (*ric*) and *bressot* (*bst*) lead to reduced or no xanthophore pigmentation.

In addition to the xanthophore pigmentation phenotype, further defects are detectable in *non blond* (*nob*) and *clorix* (*clx*) mutant larvae. Mutations in both genes lead to small otoliths (Whitfield et al., 1996). Whereas *nob* larvae are generally retarded, *clx* mutant embryos show an additional motility (Granato et al., 1996) and melanophore pigmentation defect.

**DISCUSSION**

**Genes required for xanthophore development**

In our screen for mutations with morphologically visible embryonic and larval phenotypes, we identified 17 genes required for larval xanthophore pigmentation, but without any effect on melanophores and iridophores. Multiple alleles were isolated for nine genes, ranging from two alleles (*kef*) to 14 alleles (*esr*). For eight genes only a single allele was isolated for each, leading to an average allele frequency of 3.6. From the average allele frequency of this phenotypic class we conclude that we probably identified most of the genes required specifically for xanthophore pigmentation in our screen. We might have missed additional alleles of *feta*, *tilsit*, and *yocca*, because the phenotypes of the single alleles are very weak.

**Pigment pattern formation is different in the larva and in the adult**

One surprising result of this study is the finding that most mutants with strongly reduced xanthophore pigmentation in the larva show no obvious xanthophore phenotype as adults (as observed under a dissecting microscope), indicating that larval and adult xanthophore pigmentation in the zebrafish require different genes. Mutations in only three genes (*salz*, *pfeffer* and *tartar*) cause adult phenotypes. Whereas xanthophores and melanophores are pale in *tar* homozygous adults, xanthophores are not detectable in *sal* and *pfe* homozygous adults.

Larval xanthophores in fish occupy the zones not occupied by melanophores (Epperlein and Claviez, 1982b). In *sandy* mutant larvae unpigmented melanophores form unpigmented gaps between pigmented xanthophores in the dorsal head (*sandy* in class VI.A, Fig. 6 in Kelsh et al., 1996). However, if the number of melanophores is reduced, as in *sparse* mutant larvae, xanthophores fill the vacant positions of the melanophores (*sparse* in class III, Fig. 3 in Kelsh et al., 1996). The observation of unpigmented spaces between melanophores and iridophores in *sal* and *pfe* larvae indicates that in the wild-type situation xanthophores fill the gaps between the melanophore and iridophore stripes.

Whereas xanthophores are apparently not required for the proper formation of the larval melanophore stripes, they have an important function in adult pigment pattern formation. Partial absence of xanthophores in *sal* and *pfe* heterozygous adult fish leads to irregular blue-black stripes, and a ‘salt and pepper’ pattern of melanophore pigmentation fading out towards the tail. A similar but stronger phenotype can be observed in homozygous mutant fish. The correlation of a reduced number of xanthophores with an irregular melanophore pattern in the adult is in agreement with earlier findings, which indicated that the xanthophores play an important role in pigment pattern formation in the body (Epperlein and Claviez, 1982a; Epperlein and Löbregt, 1984) and in the anal fin (Goodrich and Nichols, 1931; Goodrich et al., 1954). The correlation between xanthophore and melanophore pattern can also be seen in adult zebrafish that are mutant for *leopard*, where the number of xanthophores in the anal fin is reduced and the melanophore pigment pattern is disrupted as well (Kirschbaum et al., 1975; Haffter, personal communication). A similar influence on the adult melanophore pigment pattern by iridophores has been found (Johnson et al., 1995; Haffter, personal communication). In mutants where the number of iridophores is reduced (*transparent*, *rose*, *shady*), the melanophore pattern in the adult body is disrupted. Again this influence is restricted to the adult pigment pattern and has no effect on larval pigment pattern formation. At present it is unclear whether xanthophores have a function in the initiation or in the maintenance of the melanophore stripes. A more
detailed analysis of the transition from the larval to the adult pigment pattern in these mutants is required to address this point.

**Pteridine composition is different in bri, yob and yoc**

Mutations in three genes (edi, bri and qua) lead to the complete loss of yellow colour, whereas in all other xanthophore mutants the yellow colour is only reduced. It has been previously shown that xanthophores contain different kinds of pteridine pigments (Matsumoto and Obika, 1968; reviewed by Bagnara, 1966). They appear almost simultaneously with the differentiation of xanthophores (Kamei-Takeuchi and Hama, 1971). Specific sets of pteridines are described for many species. Most frequently xanthophores contain sepiapterine (visible yellow), drosopertene (visible red) and colourless pteridines (biopetern).

As a first step towards an analysis of the pteridine biochemistry, we performed thin-layer chromatography. We identified eight different bands that are visible under UV light. Two of these bands are visibly yellow and are therefore probably sepiapterines, whereas the others are colourless under normal illumination; no red drosopertene was found in this analysis (Matsumoto and Obika, 1968; reviewed by Bagnara, 1966). A detailed analysis will be necessary to determine which of the bands correspond to known pteridines. Mutations in three genes, bri, yob and yoc, produce similar differences in pteridine composition compared to wild type and may encode enzymes required to produce specific pteridines. Further chromatographical analysis of wild-type and mutant larvae is required to resolve the pathway for pteridine synthesis.

Mutations in two genes, edison and tartar, lead to blue fluorescence in the yolk and the entire embryo. We propose that edi and tar encode enzymes required for early steps in pteridine synthesis. The lack of edi and tar function in mutant embryos leads to the accumulation of fluorescent intermediates that, in the case of edi, seem to be toxic for the larvae. This phenotype would predict a change in pteridine composition. Surprisingly, we have not yet found a difference in the pteridine pattern for edi using chromatography. A more detailed analysis needs to be carried out to allow further characterization of the biochemical defects in these mutants.

**Yobo has similarities to the Drosophila mutation deep orange**

The yob gene product has at least two biological functions during development. The first function is in pteridine synthesis, since pteridine composition is altered in yob mutant embryos. The second function, provided maternally, is required in the embryo for normal early development. Several genes with a similar combination of zygotic and maternal requirement are known in Drosophila. For example, mutations in the deep orange (dor) gene lead to both maternal effect phenotype and a change in eye colour. The phenotype is accompanied by alterations in pteridine composition (Counce, 1957). The strength of the embryonic yob phenotype is independent of the zygotic genotype, indicating that zygotic yob function is not required during early development. This is in contrast to dor, for which expression is also required zygotically for embryonic development.

**Xanthophores and retinotectal projection**

Three mutants (esr, til and tof) were isolated with defects in xanthophore pigmentation and retinotectal axon pathfinding. Yellow pigmentation is concentrated towards the cell center in mutants of all three genes and the morphology of the xanthophores under UV light is abnormal. The distribution of the coloured organelles within the pigment cell is important for the general appearance of the body colour. Dispersed organelles lead to a strong colour, whereas pigment cells with aggregated organelles are weakly coloured (reviewed by Luby-Phelps and Schliwa, 1982; Obika, M., 1986). The pigmentation phenotype in esr, til and tof could be explained by a defect in pigment translocation. Retinotectal axons stop prematurely on their way to the tectum and show mapping defects if they reach the tectum (Karlstrom et al., 1996). The phenotypes observed in seemingly unrelated cell type suggests that esr, til and tof may encode components of the cytoskeleton. It further suggests that these genes are required in a common pathway for both pigment cell morphology and axonal pathfinding of retinal axons. This hypothesis will have to be tested by a careful analysis of the cytoskeleton in the xanthophores and the retinal axons.

In summary, the collection of mutants described here and by Kelsh et al. (1996) will be helpful tools to gain insight into different processes involved in vertebrate pigmentation.

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