Iridophore development in wild-type and periodic albino *Xenopus* larvae

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Summary. Light microscopic studies of iridophore preparations from a^p mutant *Xenopus* larvae revealed abnormalities in the morphology and size distribution of reflecting platelets; purine synthesis is normal. A pleiotropic effect of the a^p gene on melanoblasts and iridoblasts is proposed.

The periodic albino mutant (a^p) of *Xenopus* in which the differentiation of melanin pigment cells is grossly impaired[1], has also been reported to exhibit atypical iridophore development[2]. Both mutant and wild-type iridophores appear first as silvery dendritic cells on the eyes and peritoneal membranes of young larvae; but, while wild-type cells rapidly become golden and exhibit strong light-reflecting properties, mutant cells become only faintly golden and reflect light much less efficiently than their wild-type counterparts. Considerable information has accumulated recently regarding the mode of action of the a^p gene in the development of melanin pigment cells[2-7] but, little is known about mutant effect on iridophores. The common origin of melanoblasts and iridoblasts in the neural crest suggests that the mutant gene might be acting pleiotropically in affecting some aspect of differentiation common to both types of chromatoblast. As a first step in investigating this possibility, the development of mutant iridophores was examined more closely. Previous studies[8-10] have demonstrated that iridophore differentiation entails deposition of purine derivatives within membraneous organelles to form the crystalline reflecting platelets which characterize this type of pigment cell. In the present study, the mutant effect on iridophore differentiation was examined by comparing a) the types of purine and b) the morphology of reflecting platelets present in mutant and wild-type larvae.

Methods. Eggs of mutant (a^p/a^p) and wild-type ($+/+$) *Xenopus laevis* were obtained by standard methods[11]. Larvae were reared in de-chlorinated tap water and fed on a suspension of spinach.

For chromatography, purines were extracted from 12 batches of a^p/a^p and $+/+$ larvae at 2 and 6 weeks of age, using the technique described by Stackhouse[12]. Whatman No. 1 chromatographic paper was used for chromatography and chromatograms were developed ascendingly using n-propanol - 1% ammonia (2:1) as a solvent system. Purines were identified as dark absorbing spots when illuminated with shortwavelength (254 nm) UV light. The R_f-values for each purine derivative identified from the extracts were averaged and compared to the average R_f-values obtained for adenine, guanine, xanthine and hypoxanthine standards (Sigma).

For light microscopy, portions of ventral peritoneum were dissected out from a^p/a^p and $+/+$ larvae on a) day 1 (first appearance of silver iridophores in a^p/a^p and $+/+$ larvae), b) day 3 ($+/+$ iridophores now golden, a^p/a^p iridophores still silver) and c) day 10 ($+/+$ iridophores golden and strongly light-reflecting, a^p/a^p iridophores faintly golden and moderately light-reflecting). The excised tissues were mounted in Holtfreter's solution on microscope slides and examined by reflected light using a Vickers M41 Photoplan microscope. Platelets within iridophores were too closely packed for detailed examination to be made. However, iridophores in Holtfreter's solution undergo cytolysis[13], releasing populations of individual platelets into the surrounding medium. The shapes and reflecting characteristics of such platelets were noted and the sizes of platelets from a^p/a^p and $+/+$ iridophores compared by measuring the span (Feret's diameter[14]) of every platelet in representative groups of 200 platelets, using a calibrated ocular scale (each division equivalent to 0.92 µm). Platelet measurements from 5 larvae in each group were assembled to give size distribution data[15].

Results and discussion. Identical chromatograph patterns consisting of 4 purine spots were obtained from all batches of larvae regardless of age or genotype, indicating the presence of normal purine synthesis in a^p/a^p larvae. 2 spots with R_f-values of 0.53 and 0.28 were identified as hypoxanthine (R_f 0.52) and guanine (R_f 0.29), respectively. The

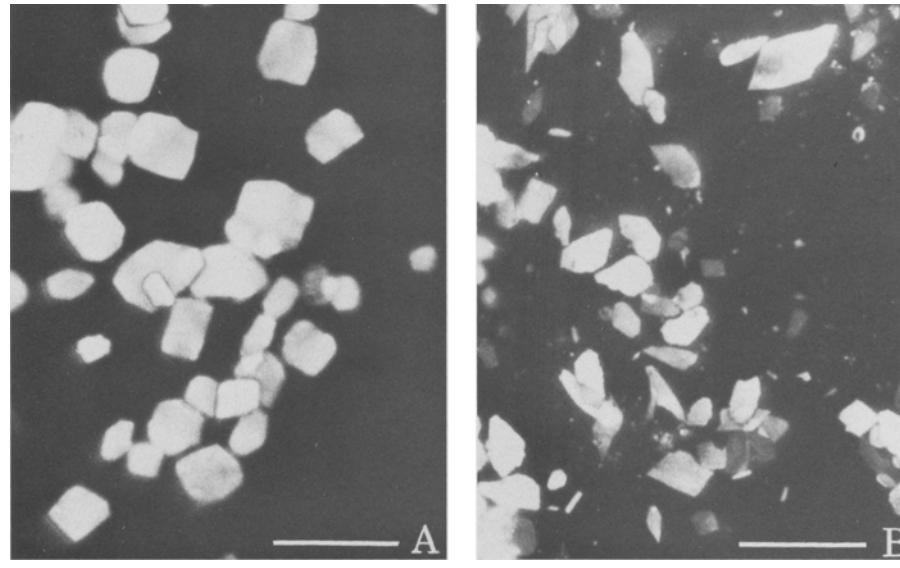

Fig. 1. Populations of reflecting platelets from $+/+$ (A) and a^p/a^p (B) iridophores on day 10 of iridophore maturation. Note the atypical morphology of a^p/a^p platelets. Reflected light. Bar: 10 µm.

other 2 spots could not be identified with certainty from the standards used, one having an R_f-value (0.65) somewhat higher than adenine (R_f 0.61) and the other (R_f 0.43) rather higher than xanthine (R_f 0.40).

Low power microscopical examination of iridophore preparations demonstrated the presence of similar amounts of reflecting material in a^p/a^p and +/+ cells. The numbers of reflecting platelets increased as iridophore differentiation progressed. High power studies revealed striking differences in the organisation of +/+ and a^p/a^p reflecting platelet populations (figure 1). Day 1 +/+ iridophores provided populations of small, square to hexagonal platelets of uniform colour and luminosity. Platelets from later +/+ iridophores were of similar character but progressively larger in size. The increase in size and number of platelets during the maturation of +/+ iridophores can be correlated to the increasing powers of light reflection and the transition from silver to gold of iridophores in situ in +/+ larvae. Platelets obtained from a^p/a^p iridophores were irregular and angular in shape and heterogeneous in colour and luminosity. Although a^p/a^p platelet populations exhibited size ranges similar to those of corresponding +/+ populations, the size distribution of platelets within each range indicated a persistent preponderance of smaller platelets (figure 2). The less efficient reflecting powers and paucity of gold coloration of maturing a^p/a^p iridophores can be accounted for in terms of the observed atypical morphology and size distribution of a^p/a^p platelets.

The findings reported here suggest that a^p/a^p iridophores have some deficiency in the processes controlling the formation of reflecting platelets. Previous studies[3,4,6] have indicated that a^p/a^p oocytes lack premelanosomes and that melanosomes synthesized in a^p/a^p larval melanoblasts are abnormal in structure. These observations support the proposal that the a^p gene has a pleiotropic effect on organelle development in both melanoblasts and iridoblasts. Further investigation of this effect may aid in establishing the developmental and cellular relationships of these pigment cells.

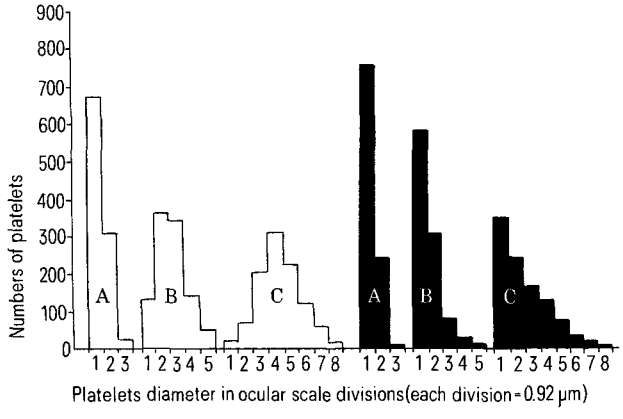

Fig. 2. Size distributions of +/+ and a^p/a^p (black hatched) reflecting platelets (1000 in each group) from day 1 (A), day 3 (B) and day 10 (C) iridophores. Note that while +/+ and a^p/a^p groups of platelets exhibit similar ranges of size, +/+ platelets tend towards maximum in the middle of each range whereas a^p/a^p platelets tend towards maximum in the lowest part of each range.

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Immunohistochemical demonstration of the loss of immunoreactive amylase from neoplastic human salivary gland[1]

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Summary. Antihuman parotid amylase antibodies raised in rabbits and horses were used as the primary antibodies in both the peroxidase-antiperoxidase and sandwich techniques for the localization of human amylase. Immunoreactive enzyme was demonstrated in the normal acinar cells of salivary glands and pancreas. Malignant transformation, which has occasionally resulted in ectopic production of amylase by various tissues, actually caused a loss of amylase synthesis by the transformed acinar cells of salivary glands and did not result in elaboration of amylase by transformed ductal cells.

Comparative peptide mapping studies indicate that human salivary and pancreatic amylases (Amy_1 and Amy_2) differ but slightly in amino acid sequence[3,4]. This should make it possible to use antibody raised against one or the other amylase for immunohistochemical localization of either. Ectopic production of amylase has been reported for certain tumors[5-9]. These tumor amylases closely resemble the salivary enzyme[4]. Several authors have used these properties of amylase and its antibody for immunofluorescent[11,12] and ultrastructural[12] localization of the enzyme. We report here results on localization of amylase in human salivary glands and pancreas, the loss of amylase expression by transformed acinar cells and lack of induction of amylase production in neoplastic ductal cells in human parotid gland tumors.

Materials and methods. Antihuman salivary amylase antibody was prepared in rabbits and horses. The IgG fraction of rabbit serum was isolated by ammonium sulfate precipi-