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Experimental evidence for autonomous action of the periodic albinism (a^p) gene within developing retinal pigment cells and melanophores of *Xenopus laevis*

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SUMMARY

Genes which affect pigment elaboration may do so by autonomous action within the developing pigment cells or by way of tissue interactions leading to pigment cell differentiation. The site of action of the periodic albinism (a^p) gene was investigated by substituting presumptive neural ectoderm of gastrulae of one genotype with uncommitted ectoderm of different genotype. Retinal pigment cells and melanophores arising from such grafts were found to differentiate according to their own genotype in spite of having spent their entire developmental history in tissues of different genotype. This finding demonstrates autonomous action of the a^p gene within pigment cell derivatives and does not support recent proposals that the a^p gene is involved in inductive interactions leading to melanogenesis. Experiments in which portions of presumptive dorsal mesoderm, implanted in gastrulae of different genotype, induced secondary pigment cells of host phenotype further support the proposal that the a^p effect on pigment cells is not mediated by inductive interactions.

INTRODUCTION

The periodic albino mutant (a^p/a^p) of *Xenopus laevis* is characterized by deficiencies in pigment cell development. Mutant oocytes and embryos completely lack melanin and post-metamorphic animals possess a typical albinotic phenotype. During larval stages, the retinal pigment epithelium (RPE) and melanophores exhibit a delayed, very limited elaboration of melanosomes which degenerate later in larval life (Hoperskaya, 1975). The iridophores of a^p/a^p larvae are also abnormal, being less iridescent in mutant than in wild-type (+/+) larvae (MacMillan, 1979).

A number of intrinsic and extrinsic factors can influence the differentiation of pigment cells. Hence genes which affect pigment cell differentiation could be acting at any one of several levels. Attempts have been made to establish which properties of developing pigment cells are affected in the mutant. Studies on the incidence of melanophores in ventral trunk tissues, isolated at progressively later stages of neural crest migration, and counting of DOPA-positive cells have

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shown that the migration of melanoblasts and numbers of these cells colonizing embryonic tissues are normal in the mutant (unpublished results). Biochemical assays have indicated that tyrosinase, the key enzyme involved in melanogenesis, is present in greater amounts than normal in mutant oocytes and larval cells (Wyllie & de Robertis, 1976; Tompkins, Knight & Burke, 1977). Ultrastructural observations have demonstrated that premelanosomes are absent in mutant oocytes (Bluemink & Hoperskaya, 1975) and that melanosomes synthesised during larval stages in mutant pigment cells are abnormal in structure (Hoperskaya, 1978). The a^p gene, therefore, appears to be involved in processes leading to premelanosome assembly. Further evidence that the a^p gene is involved in organelle development is provided by a report that the reflecting platelets of mutant iridophores are disorganized with respect to size and shape (MacMillan & Gordon, 1981).

Attempts have also been made to determine whether the a^p gene acts autonomously within developing pigment cells or acts indirectly by way of tissue interactions involved in pigment cell differentiation. Hoperskaya (1978) and Hoperskaya & Golubeva (1980) have proposed that the mutant effect on RPE is due to a deficiency in a specific inducing factor for melanogenesis which in $+/+$ embryos is synthesized by the anterior dorsal mesoderm of gastrulae and later by the head endomesoderm of tail-bud stages. She has further proposed that the competence of RPE to synthesize melanosomes in response to the inducing factor is lost by late tail-bud stages. While these proposals appear capable of explaining the a^p gene effect on RPE they cannot account for certain known features of melanophore development in mutant and wild-type larvae. Reciprocal transplantations of neural crest between a^p/a^p and $+/+$ embryos (Hoperskaya, 1978) and culture of a^p/a^p and $+/+$ neural crest in tissues of different genotype (MacMillan, 1979) have indicated that the mutant effect on melanophores and iridophores is intrinsic in neural crest cells and is not mediated by chromatoblast-tissue interactions. The possibility that melanogenic inducers act prior to neural crest formation seems unlikely since there is considerable evidence that neural crest cells are pluripotent, their final determination as pigment cells depending on factors arising either during their migration or after their localization in embryonic tissues (see e.g. Noden, 1978 and Bagnara *et al.* 1979). Furthermore, the melanophore derivatives of the neural crest retain their competence for melanogenic induction throughout larval and adult life (Pehleman, 1972; Bagnara & Hadley, 1973). The proposal that the a^p phenotype is mediated by an inducer of melanogenesis also fails to explain the pleiotropic effect of the a^p gene on iridophores (MacMillan, 1979).

Further investigation of the site of expression of the a^p gene seemed necessary in order to clarify these anomalies. Neural derivatives including neural crest and optic cup develop from the inner layer of uncommitted ectoderm as a result of primary induction by the dorsal mesoderm of the archenteron roof (Spemann

& Mangold, 1924). These tissues are brought together during gastrulation as a result of morphogenetic movements. In the present study, autonomous action of the a^p gene in pigment cell development was investigated by transplanting blastocoele roof ectoderm from gastrulae of one genotype to a position overlying the dorsal mesoderm of gastrulae of different genotype. Isolated blastocoele roof ectoderm can only give rise to epidermal structures but under the influence of dorsal mesoderm will form neural and pigment cell derivatives (Slack & Forman, 1980). Hence pigment cells arising from grafted ectoderm would be of donor genotype but would have undergone their entire development in tissues of host genotype. The presence of pigment cells of donor genotype would indicate autonomous action of the mutant gene in pigment cell development while the absence of such cells would demonstrate the involvement of tissue interactions in expression of the a^p phenotype. The possibility that expression of the a^p phenotype is mediated by tissue interactions occurring during primary induction was investigated by implanting prospective dorsal mesoderm of one genotype into the blastocoeles of gastrulae of different genotype. Such implants are known to result in the formation of secondary embryonic axes, the pigment cell components of which arise from induced host ectoderm (Spemann & Mangold, 1924). Hence any involvement of the a^p gene in tissue interactions occurring during primary induction would be indicated by the presence of pigment cells of donor phenotype in the secondary tissues. These experiments demonstrate unequivocally that the a^p gene is not involved in tissue interactions leading to pigment cell differentiation but acts autonomously within developing pigment cells.

MATERIALS AND METHODS

Eggs of a^p/a^p and $+/+$ *Xenopus laevis* were obtained by standard methods (New, 1966). The mutant frogs were obtained from Dr J. B. Gurdon (MRC Laboratory of Molecular Biology, Cambridge) whose stock is derived from the original a^p mutant strain discovered by Dr O. Hoperskaya in Moscow. The techniques used in preparing and culturing embryos have been discussed previously (MacMillan, 1976).

Heterotopic transplantation of blastocoele roof ectoderm to the dorsal surface of gastrulae of different genotype

Square-shaped portions of ectoderm were removed from the blastocoele roofs of stage-10 $+/+$ and a^p/a^p gastrulae and transplanted to previously prepared sites on the dorsal surfaces of gastrulae of the same age but of different genotype (Fig. 1A, B staging according to Nieuwkoop & Faber, 1967). The size of the grafts and their position on host embryos were selected to ensure, as far as possible, that the inner ectoderm layer of the grafts would participate in the formation of both the optic cup and anterior neural crest of one side of the

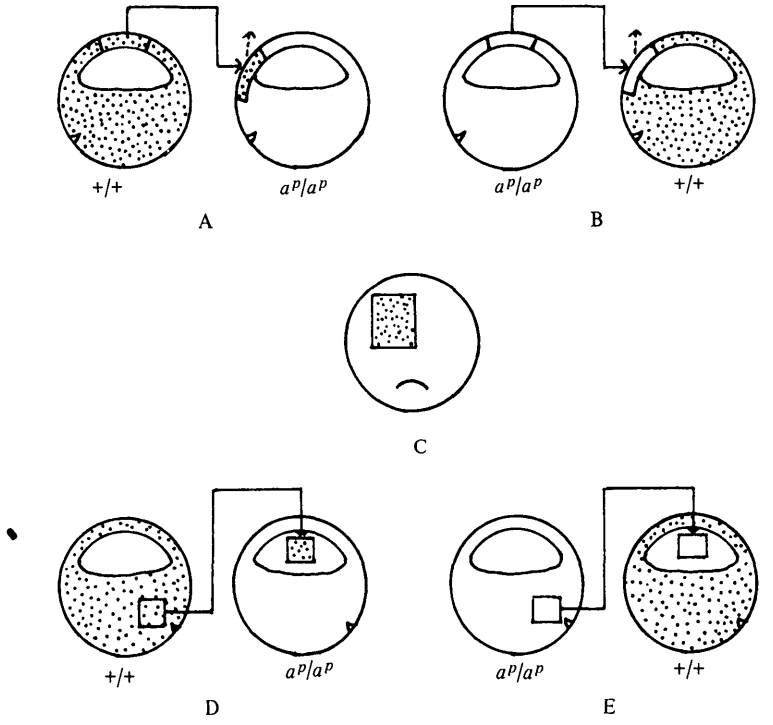

Fig. 1. Experimental procedures using stage-10 gastrulae. (A, B) Heterotopic transplantation of presumptive ectoderm of one genotype to presumptive neural area of gastrula of different genotype. (C) Position of transplant on host gastrula. (D, E) Implantation of presumptive dorsal mesoderm of one genotype into blastocoele of gastrula of different genotype.

embryo (Fig. 1C). Twenty a^p/a^p and 18 + / + host embryos continued to develop until stage 40–41 at which time the phenotypic characteristics of the pigment cells were noted.

Implantation of prospective dorsal mesoderm into the blastocoele of gastrulae of different genotype

Portions of prospective dorsal mesoderm were removed from the deep marginal zones of stage 10 + / + and a^p/a^p gastrulae and implanted, by way of an incision in the blastocoele roof, into the blastocoele of gastrulae of the same age but of different genotype (Fig. 1 D, E). Sixteen a^p/a^p and 14 + / + host embryos continued to develop until stage 40–41 at which time the phenotypic characteristics of pigment cells associated with secondary tissues were noted.

Both the above sets of experiments were controlled by normally developing

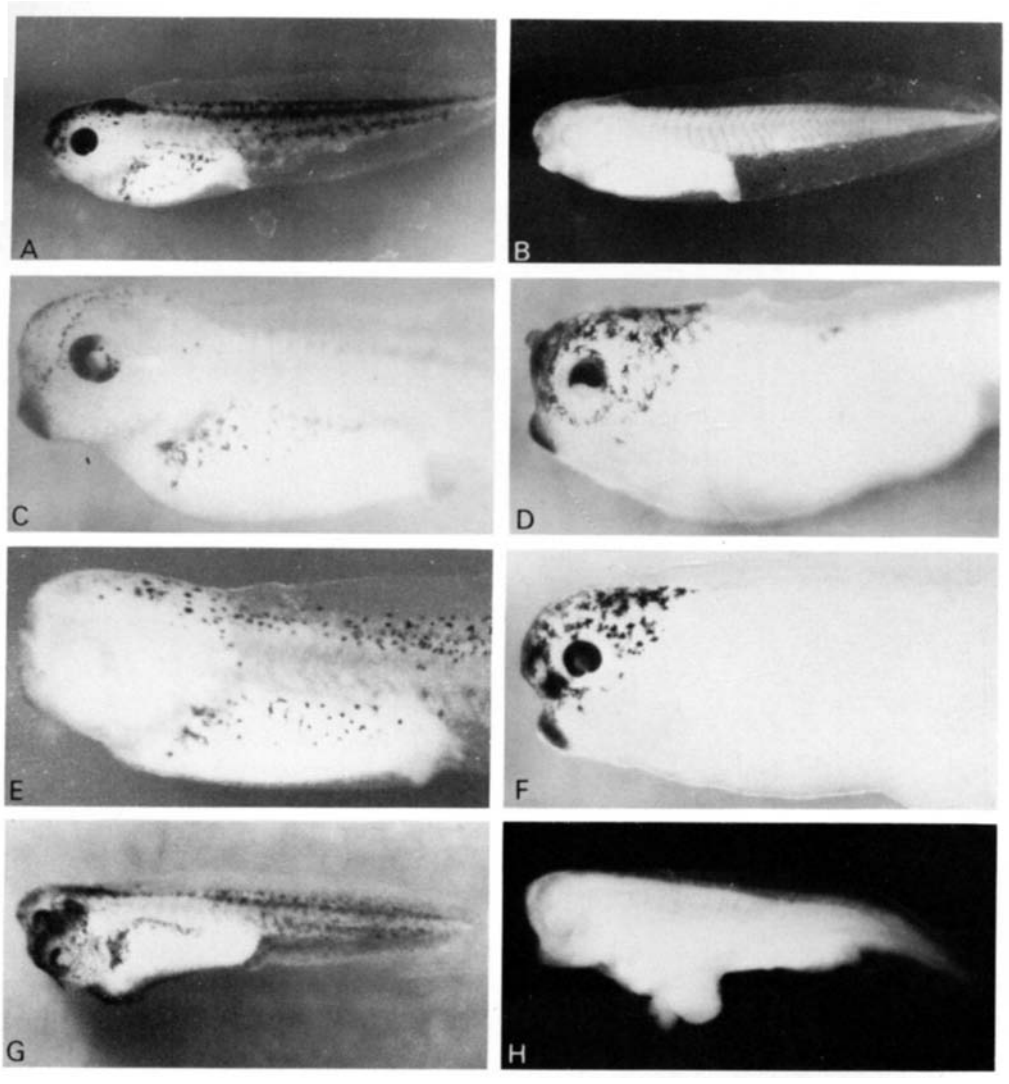

Fig. 2. Pigmentation characteristics of control and experimental host larvae. (A) Stage-41 +/+ control larva. (B) Stage-41 a^p/a^p control larva. (C, E) Stage-41 +/+ host larvae to which a^p/a^p presumptive ectoderm had been transplanted at gastrula stage 10. The RPE is partially (C) or entirely (E) of a^p/a^p phenotype and melanophores are entirely (C) or partially (E) absent from head tissues. (D, F) Stage-41 a^p/a^p host larvae to which +/+ presumptive ectoderm had been transplanted at gastrula stage 10. The RPE is partially (D) or entirely (F) of +/+ phenotype and +/+ melanophores are present in head tissues. (G) Stage-40 +/+ larva exhibiting a secondary head, induced by implanted a^p/a^p dorsal mesoderm. Note the +/+ pigmentation characteristics of the secondary tissues. (H) Stage-40 a^p/a^p larva exhibiting a secondary head, induced by implanted +/+ dorsal mesoderm. Note the a^p/a^p pigmentation characteristics of the secondary tissues.

+/+ and a^p/a^p embryos from the same egg batches. The fate maps of Keller (1975, 1976) were used as a guide when excising prospective tissues.

RESULTS

The characteristic features of pigment cell development in stage-41 +/+ and a^p/a^p larvae are demonstrated in Fig. 2A, B.

Heterotopic transplantation of blastocoele roof ectoderm to the dorsal surface of gastrulae of different genotype

(*a*) a^p/a^p *grafts on* +/+ *hosts.* At stages 40–41 the heads of all host larvae exhibited areas from which melanophores were absent. These gaps in pigmentation were apparent only on the side of the larva bearing the graft and were considered to represent areas which had been colonized by amelanotic a^p/a^p melanophores. In addition, the RPE of eyes on the side bearing the graft was entirely (44·44 % of larvae) or partially (55·56 %) of a^p/a^p phenotype (Fig. 2C, E).

(*b*) +/+ *grafts on* a^p/a^p *hosts.* At stage 40–41 the heads of all host larvae exhibited melanophores of +/+ phenotype. These cells were most numerous on the side of the larva bearing the graft. In addition, the RPE of eyes on the side bearing the graft was entirely (60 % of larvae) or partially (40 %) of +/+ phenotype (Fig. 2D, F).

Occasionally eyes on the side opposite the grafts also exhibited RPE which was partially of donor phenotype. This finding is explicable in terms of (*a*) an inevitable variation in the topographical location of graft ectoderm and hence in the extent to which it had participated in RPE formation in one or both eyes or (*b*) a previous report (Jacobson & Hirose, 1978) that cells from one side of the neural plate contribute to the contralateral optic lobe.

Implantation of prospective dorsal mesoderm into the blastocoele of gastrulae of opposite genotype

At stage 40–41 all experimental larvae possessed ventrally situated secondary tissues. In 43·75 % of a^p/a^p and 35·63 % of +/+ hosts the secondary tissues were composed of head, or head and trunk components. The RPE and melanophores associated with these tissues were entirely of host phenotype; that is, a^p/a^p host larvae containing +/+ dorsal mesodermal implants exhibited only a^p/a^p pigment cells, while +/+ host larvae containing a^p/a^p dorsal mesodermal implants exhibited only +/+ pigment cells (Fig. 2G, H). The remaining larvae possessed only secondary trunk or trunk and tail tissues and in these larvae, also, the melanophores were entirely of host phenotype.

DISCUSSION

The expression of a^p/a^p and $+/+$ phenotypes was found to depend entirely on the genotype of the ectoderm from which the pigment cells were derived. RPE and melanophores arising from grafts of uncommitted gastrula ectoderm differentiated according to their own genotype in spite of having spent their entire developmental history in tissues of different genotype. This result indicates that the a^p gene acts autonomously during pigment cell differentiation and is not involved in the tissue interactions which lead to the determination and differentiation of these cells. This finding is at variance with reports by Hoperskaya (1978) and Hoperskaya & Golubeva (1980) that the a^p gene is involved in a sequential induction of melanogenesis in RPE, firstly by anterior dorsal mesoderm and later by head endomesoderm. These proposals were based on two sets of results which appear to be open to equivocal interpretation. In one set of experiments Hoperskaya (1978) found that reciprocal transplantation of optic vesicles between early tailbud $+/+$ and a^p/a^p embryos often resulted in RPE which was partially of host phenotype. She interpreted this result as indicating that the host-type component represented RPE of donor genotype whose differentiation had been influenced by the endomesodermal tissues of the host. Unfortunately the experimental procedures used did not exclude the possibility that the host-type component might represent a later contribution of host tissues to the eye. This possibility is supported by recent experiments by Gaze, Feldman, Cooke & Chung (1979), who found that rotated grafts of complete $+/+$ optic vesicles transplanted homotopically to a^p/a^p embryos at early tailbud stages gave rise to eyes in which the RPE was partially of albino phenotype. Retinotectal mapping of such eyes indicated that, whereas the $+/+$-type component gave a rotated map in keeping with the rotation of the donor eye, the a^p/a^p-type component gave an unrotated map indicating its derivation from host tissues. This finding strongly suggests that in Hoperskaya's experiments the RPE of host phenotype present in transplanted eyes represented a contribution of host tissues to the eye and did not indicate a^p gene dependent induction of melanogenesis by host tissues. In a further set of experiments Hoperskaya & Golubeva (1980) found that reciprocal transplantation of dorsal ectoderm between $+/+$ and a^p/a^p stage-10 gastrulae gave rise to eyes in which the RPE was entirely or mainly of host phenotype. This finding, which suggests a^p gene involvement in tissue interactions leading to melanogenesis, does not agree with the results obtained in the present experiments in which ectodermal grafts to the dorsal region of stage-10 embryos gave rise to eyes of donor phenotype. This anomaly can perhaps be accounted for in terms of differences in the experimental procedures used. The ectodermal grafts in Hoperskaya's experiments appear to have been of a size which was just capable of replacing the anterior-most neural plate on one side of the embryo. The criterion used to indicate whether or not such grafts would participate in eye formation was

apparently the position occupied by graft superficial ectoderm at open neural plate stage 17. However, the position of superficial ectoderm at stage 17 may not exactly correspond to the present position of the inner ectoderm cells (from which the eye develops) which underlay it at stage 10. Indeed, a recent report by Keller (1980) indicates that during gastrulation (between stages 10 and $11\frac{1}{2}$) epibolic movements of the superficial ectoderm do not match those of the inner layer which spreads further and more quickly than the superficial layer. It is possible, then, that in Hoperskaya's experiments the inner ectoderm of grafts transplanted at stage 10 could have been displaced by host cells which proceeded to participate in optic vesicle formation. In the present experiments the problem of assessing the exact position of prospective eye and neural crest tissues at stage 10 was solved by using grafts which were considerably larger than the prospective tissues which they were intended to replace.

The present proposal that the a^p gene is not involved in tissue interactions leading to melanogenesis is further supported by (*a*) the present finding that implants of inducer mesoderm of one genotype were completely unable to alter the capacity for melanogenesis of RPE and melanophores arising from host ectoderm of different genotype, (*b*) previous demonstrations that the expression of the a^p gene is intrinsic in neural crest cells (Hoperskaya, 1978; MacMillan, 1979) and (*c*) experiments carried out in this laboratory (unpublished results) to test the possibility that the a^p gene might affect permissive induction of RPE by the neural crest derived ectomesenchyme which surrounds the optic vesicle (see Lopashov, 1963); reciprocal exchange of anterior neural fold between a^p/a^p and $+/+$ embryos had no effect on the differentiation of host RPE.

It can be concluded that the a^p gene acts autonomously within both RPE and melanophores, and that previous reports of a^p gene effects on tissue interactions are erroneous. The precise role of the a^p gene in melanosome differentiation is unclear. It may code directly for structural proteins of the melanosome matrix or act in regulating the production of such proteins. The transitory formation of abnormal melanosomes during larval stages in the mutant has been shown to depend on a timely environmental contribution which has been suggested to act by substituting for the defective gene product in premelanosome assembly (MacMillan, 1980). The pleiotropic effect of the a^p gene on the size and shape of iridophore platelets (MacMillan & Gordon, 1981) suggests that the a^p gene is operating in both chromatoblast types but whereas the gene product is essential for melanosome formation it plays a less vital role in iridophore platelet formation. Experiments designed to further elucidate a^p gene action are currently being undertaken.

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