J. Embryol. exp. Morph. Vol. 36, 3, pp. 555–559, 1976
Printed in Great Britain

High tyrosinase activity in albino *Xenopus laevis* oocytes

By A. H. WYLLIE[1] AND E. M. DE ROBERTIS[1]
From the MRC Laboratory of Molecular Biology, Cambridge

SUMMARY

Tyrosinase was measured in oocytes of the recently described albino mutant (a^pa^p) of *Xenopus laevis*. Although these oocytes show no pigmentation and the eggs are known to contain no melanosomes, tyrosinase – which is probably the only enzyme necessary for melanin synthesis from tyrosine – was increased more than twofold relative to the wild type. Tyrosinase recovered from albino and wild type oocytes showed the same K_M with respect to tyrosine, and this was not altered by previous gonadotrophin stimulation *in vivo*. The tyrosinase assay, based on [^{14}]tyrosine incorporation into acid-insoluble products, was of greater sensitivity than previously described methods of the same type, through removal of low molecular weight material from the oocyte homogenate prior to incubation, and the use of tyrosine of high specific activity.

INTRODUCTION

Periodic albinism is a recently described recessive mutant (a^pa^p) of *Xenopus laevis* (Hoperskaya, 1975). The precise biochemical defect is not yet known. Throughout most of their lifespan affected animals are almost completely free of melanin pigmentation but it is clear that melanin synthesis is possible in at least some cells since pigment appears transiently in the tadpole and persists, albeit in a very few sites, even in the adult. Mutant oocytes and eggs, unlike the wild type, remain pigment-free throughout their development; in the electron microscope they show no premelanosomes or melanosomes but in all other respects appear normal (Bluemink & Hoperskaya, 1975).

It seemed desirable to attempt to identify the biochemical defect in periodic albinism because the mutant might prove useful in the study of the control of gene expression in the oocyte. Although many different genes are believed to be transcribed in oocytes (Callan & Lloyd, 1960), tyrosinase – probably the only enzyme required for the synthesis of melanin – is one of the few clearly identified protein products of oocyte-active genes. In this paper we report unexpectedly high levels of tyrosinase in this mutant, using a sensitive radiometric assay.

[1] *Authors' address:* MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

MATERIALS AND METHODS

Oocytes were obtained from previously untreated wild-type and albino frogs, and also from frogs which had received approximately 4 i.u./g chorionic gonadotrophin (Chlorulon, Intervet) 3 days previously. Tyrosinase was assayed bya modification of the method of Achazi & Yamada (1972). Batches of between 100 and 250 oocytes, graded by size, were homogenized in 0·1 M phosphate buffer, pH 6·8, solubilized with 1 % Triton X-100 and centrifuged at 70000 ***g*** for 30 min (Eppig & Dumont, 1974). Low molecular weight material was removed from the supernatant on a Sephadex G25 column (7 mm diameter × 130 mm), since this was found to enhance enzyme product accumulation around 20-fold. Protein concentration in the eluate (Lowry, Rosebrough, Farr & Randall, 1951) was adjusted so that each assay tube contained 50 μg; incubation was at 25 °C for 16 h in a total volume of 120 μl 0·1 M phosphate buffer, pH 6·8, together with 4×10^{-6} M L-tyrosine, 4×10^{-6} M L-dihydroxyphenylalanine (dopa), 50 μg/ml cycloheximide, 200 i.u./ml penicillin G, 200 μg/ml streptomycin and 0·1 μCi L-[U-^{14}C]tyrosine (sp. act. 495 mCi/mmol; Amersham). Material insoluble in 10 % TCA was collected on 2·4 cm diameter Whatman 3MM filter paper discs and counted with an efficiency of approximately 60 % using Liquifluor (New England-Nuclear) as scintillant. These conditions were modified as follows in experiments to evaluate apparent K_M. Portions of whole ovary were homogenized, and the Sephadex G25 eluate was incubated with a range of tyrosine concentrations at either 50 or 100 μg per assay. Duration of incubation was chosen from 5 to 16 h such that substrate incorporation was linear throughout.

Tyrosinase activity showed an absolute requirement for L-dopa in the assay mixture, and was inhibited both by heating to 80 °C for 5 min and by 0·66 mM 1-phenyl-2-thiourea, a specific tyrosinase inhibitor (Whittaker, 1966), although phenylthiourea was usually less effective than heat inactivation.

RESULTS

Extracts of unstimulated oocytes from both albino and wild-type frogs contained tyrosinase activity; indeed expressed per μg protein, or per oocyte, the albino oocytes showed more activity than the wild type (Table 1).

In contrast to one previous report in which low molecular weight material was not removed from the homogenate prior to assay (Eppig & Dumont, 1974) the current assay recorded tyrosinase activity in mature oocytes (diameter more than 1000 μm) as well as in the immature (diameter 500–700 μm). In the wild type, enzyme activity per oocyte was the same in immature and mature oocytes, whilst in the albino enzyme activity per oocyte increased with maturity (Table 1).

Measurements of the apparent K_M for tyrosine showed that albino and wild type enzyme behaved identically, and this was not altered by previous gonadotrophin stimulation (Fig. 1).

Table 1. *Tyrosinase activity in unstimulated* a^p/a^p *and wild-type oocytes*

	Oocyte		Tyrosinase*	
Type	Diameter (μm)	Total protein (μg)	Per μg protein	Per oocyte
a^p/a^p	500–700	11·8	145	1711
Wild	500–700	11·0	50	549
a^p/a^p	> 1000	40·1	83	3339
Wild	> 1000	37·6	14	523

* pmoles tyrosine converted to insoluble product per hour. All figures derive from the mean of three estimations minus heat-inactivated controls. Approximately 4 small or 1·3 large oocytes are represented per assay tube, being aliquots from batches of more than 100 oocytes.

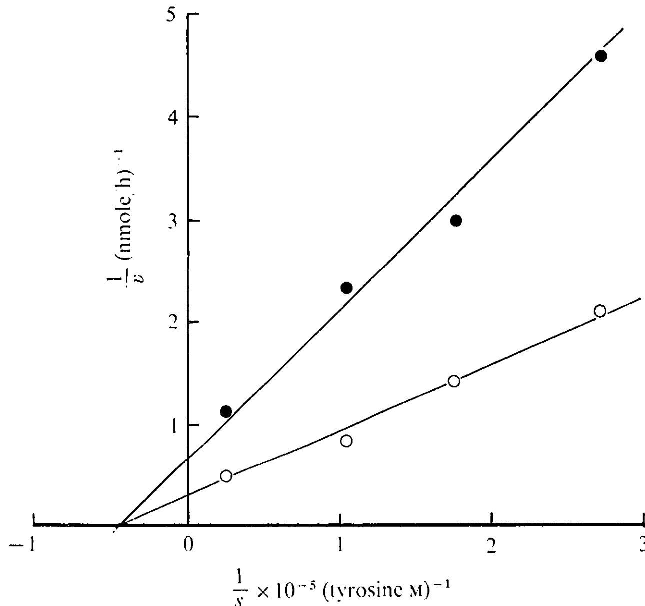

Fig. 1. Representative reciprocal plots of tyrosinase activity, with respect to tyrosine concentration in ovary homogenates from albino (○) and wild type (●) frogs treated with gonadotrophin. Each point derives from the mean of three estimations minus heat-inactivated controls. In repeated experiments the mean K_M was found to be $3{\cdot}0 \times 10^{-5}$ M for both gonadotrophin-treated albino (four experiments) and gonadotrophin-treated wild type (two experiments). Single experiments using albino and wild-type ovary from untreated frogs were also consistent with a common K_M of around 3×10^{-5} M.

DISCUSSION

Tyrosinase appears to be essential for melanogenesis *in vivo*: there are several examples of loss of pigmentation associated with relative or absolute inactivity of the enzyme (Whittaker, 1967; Benjamin, 1970; Pawelek, Wong, Sansone &

Morowitz, 1973) and of increased activity preceding the appearance of pigmentation in previously amelanogenic cells (Pawelek *et al.* 1973; Benson & Triplett, 1974). Nonetheless there are precedents for the existence of tyrosinase without concurrent melanin synthesis. Firstly, tyrosinase has been found in melanin-free organelles of pigment cells, a finding possibly explicable on the basis of restricted permeability of these organelles to tyrosine (Seiji & Iwashita, 1965; Whittaker, 1973; Varga *et al.* 1976). Secondly there is evidence that some cells contain tyrosinase in inactive form, either as a larger molecule requiring activation by a protease (Barisas & McGuire, 1974; Benson & Triplett, 1974), or because of association with a specific inhibitor (Seiji *et al.* 1973). This type of mechanism has been invoked to explain a tyrosinase-containing albino mutant of *Rana pipiens* (Smith-Gill, Richards & Nace, 1972).

Whilst the present experiments do not exclude any of these possibilities it is unlikely that the relatively mild homogenization procedures used would have allowed significant activation of proenzyme or removed protein inhibitors of the type described by Seiji *et al.* (1973). Moreover, the absence of premelanosomes, as in the *Xenopus* a^p/a^p mutant (Bluemink & Hoperskaya, 1975), is unusual in albinism; more commonly immature melanosomes containing a characteristic banded protein matrix are present, but tyrosinase activity is low (Benjamin, 1970; Theriault & Hurley, 1970; Moellmann, McGuire & Lerner, 1973). It is possible that in *Xenopus* periodic albinism the defect lies not in tyrosinase but in other proteins involved in melanosome assembly. The relatively high tyrosinase activity in the albino might then be due to absence of the normal intramelanosomal 'masking' of the enzyme by melanin and protein deposition around it (Seiji & Miyazaki, 1971).

We are most grateful to Dr J. B. Gurdon for laboratory facilities, guidance and encouragement, and to Drs R. A. Laskey and G. A. Partington for comments on the text. A. H. Wyllie is supported by a Michael Sobell Fellowship of the Cancer Research Campaign, and E. De Robertis by a fellowship of the Jane Coffin Childs Memorial Fund for Medical Research.

REFERENCES

Achazi, R. & Yamada, T. (1972). Tyrosinase activity in the Wolffian lens regenerating system. *Devl Biol.* **27**, 295–306.

Barisas, B. G. & McGuire, J. S. (1974). A proteolytically activated tyrosinase from frog epidermis. *J. biol. Chem.* **249**, 3151–3156.

Benjamin, C. P. (1970). The biochemical effects of the *d*, *m* and *a* genes on pigment cell differentiation in the axolotl. *Devl Biol.* **23**, 62–85.

Benson, S. C. & Triplett, E. L. (1974). The synthesis and activity of tyrosinase during development of the frog *Rana pipiens*. *Devl Biol.* **40**, 270–282.

Bluemink, J. G. & Hoperskaya, O. A. (1975). Ultrastructural evidence for the absence of premelanosomes in eggs of the albino mutant (a^p) of *Xenopus laevis*. *Wilhelm Roux' Archiv. EntwMech. Org.* **177**, 75–79.

Callan, H. G. & Lloyd, L. (1960). Lampbrush chromosomes of crested newts. *Phil. Trans. R. Soc.* B **243**, 135–219.

Eppig, J. J. & Dumont, J. N. (1974). Oogenesis in *Xenopus laevis* (Daudin). II. The induction and subcellular localisation of tyrosinase activity in developing oocytes. *Devl Biol.* **36**, 330–342.

Hoperskaya, O. A. (1975). The development of animals homozygous for a mutation causing periodic albinism (a^p) in *Xenopus laevis*. *J. Embryol. exp. Morph.* **34**, 253–264.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

Moellmann, G., McGuire, J. & Lerner, A. B. (1973). Intracellular dynamics and the fine structure of melanocytes with special reference to the effects of MSH and cyclic AMP on microtubules and 10-μm filaments. *Yale J. Biol. Med.* **46**, 337–360.

Pawelek, J., Wong, G., Sansone, M. & Morowitz, J. (1973). Molecular controls in mammalian pigmentation. *Yale J. Biol. Med.* **46**, 430–443.

Seiji, M., Fukuzawa, H., Miyazaki, N., Akiba, H. & Kato, T. (1973). Regulatory mechanisms of tyrosinase activity in melanocyte. I. *Yale J. Biol. Med.* **46**, 508–515.

Seiji, M. & Iwashita, S. (1965). Intracellular localization of tyrosinase and site of melanin formation in melanocytes. *J. invest. Derm.* **45**, 305–314.

Seiji, M. & Miyazaki, K. (1971). Melanization and tyrosinase activity. *J. invest. Derm.* **57**, 316–322.

Smith-Gill, S., Richards, C. & Nace, G. (1972). Genetic and metabolic basis of two 'albino' phenotypes in the leopard frog, *Rana pipiens*. *J. exp. Zool.* **180**, 157–168.

Theriault, L. L. & Hurley, L. S. (1970). Ultrastructure of developing melanosomes in *C57 black* and *pallid* mice. *Devl Biol.* **23**, 261–275.

Varga, J. M., Moellmann, G., Fritch, P., Godawska, E. & Lerner, A. B. (1976). Association of cell surface receptors for melanotropin with the Golgi region in mouse melanoma cells. *Proc. natn. Acad. Sci. U.S.A.* **73**, 559–562.

Whittaker, J. R. (1966). An analysis of melanogenesis in differentiating pigment cells of ascidian embryos. *Devl Biol.* **14**, 1–39.

Whittaker, J. R. (1967). Loss of melanotic phenotype *in vitro* by differentiated retinal pigment cells: demonstration of mechanisms involved. *Devl Biol.* **15**, 553–574.

Whittaker, J. R. (1973). Tyrosinase in the presumptive pigment cells of Ascidian embryos: tyrosine accessibility may initiate melanin synthesis. *Devl Biol.* **30**, 441–454.

(*Received 15 June 1976*)