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Common Origin of Pigment Cells

Joseph T. Bagnara, Jiro Matsumoto, Wayne Ferris,
Sally K. Frost, William A. Turner, Jr.,
T. T. Tchen, John D. Taylor

Summary. The fundamentally diverse vertebrate pigment cells, melanophores, xanthophores, and iridophores, contain pigmentary organelles known, respectively, as melanosomes, pterinosomes, and reflecting platelets. Their pigments are melanins, pteridines, and purines. Mosaic pigment cells containing more than one type of organelle have been observed and mosaic organelles containing more than one type of pigment have been discovered. It is proposed that the various pigment cells are derived from a stem cell that contains a primordial organelle of endoplasmic reticular origin. This primordial organelle can differentiate into any of the known pigmentary organelles.

The neural crest of vertebrate embryos is intriguing because of the diversity of cell types to which it gives rise (*1*). Among these derivatives are pigment cells, elements of the peripheral nervous systems including the sensory and autonomic ganglia, cartilage, skeletal and connective tissue, and endocrine cells such as the calcitonin-secreting cells of the thyroid and ultimobranchial bodies. It seems implicit from their similar origin that even the most different of these neural crest derivatives must possess common features that set them apart from other elements in the hierarchy of differentiation, but so far none have been identified. In an attempt to approach this problem we have focused our attention on one group of neural crest derivatives, the chromatophores (melanophores, xanthophores, iridophores), which are fundamentally distinct from one another (*2*). It should be emphasized that, while it is generally understood that epidermal melanophores are derived from the neural crest, the same is true for dermal chromatophores including melanophores, xanthophores, and iridophores. Much new information has been obtained about the ultrastructure, chemical composition, and development of these various pigment cells, and this new knowledge has led to a better understanding of how closely the seemingly diverse pigment cells are actually related to one another (*3*). One example of this close relationship is the existence of mosaic pigment cells. The discovery of such cells formed the basis of an hypothesis that the diverse pigment cells of vertebrates originate from a stem cell of neural crest origin (*4*). This is thought to be accomplished by the appropriate differentiation of a primordial organelle into any of the specific pigmentary organelles characteristic of the various pigment cells. Thus, a melanin-containing melanosome, a purine-containing reflecting platelet, or a pteridine-containing pterinosome could be generated to form respectively, melanophores, iridophores, or xanthophores.

In order to fully comprehend the developmental latitude that must be attributed to the stem cell, it is necessary to understand how different are the various pigmentary organelles in both form and composition (Figs. 1 to 5) (*2*). In most vertebrates, melanosomes of both dermal and epidermal melanophores (-cytes) are electron-opaque ellipsoidal structures about 5 nanometers in diameter that usually contain the black-brown insoluble eumelanins. In contrast, melanosomes found in epidermal melanophores in the integument of some birds and mammals, including human red hair, contain phaeomelanin, a lighter colored sulfur-bearing melanin. The phaeomelanosome is about 3 nm in diameter and is somewhat amorphous. Reflecting platelets are the flat-appearing organelles of iridophores. They usually appear as stacks of empty spaces that were previously occupied by purine crystals lost during staining and sectioning. Their purine constituents, notably guanine, hypoxanthine, adenine, and uric acid do not serve as true pigments, but are involved in imparting structural colors. The pigmentary organelles of xanthophores and erythrophores are called pterinosomes. They are spherical in form, about 5 nm in diameter, and contain an internal series of concentric lamellae. It is not known where the monomeric and dimeric pteridines found in these organelles are localized. While the various fully differentiated pigmentary organelles stand out in marked contrast to one another, their early stages of organellogenesis are similar, and they seem to be derived from the endoplasmic reticulum (*3*). In fact, it is this similarity in origin that provides a major part of the concept we are developing concerning the existence of a primordial organelle.

An implicit aspect of our hypothesis concerning the differentiation of the various specific chromatophore types from a stem cell, in response to cues present in the tissue milieu, is that irrevocable chromatophore determination does not take place at the neural crest stage. It is

Joseph T. Bagnara and Wayne Ferris are professors of biology at the University of Arizona, Tucson 85721, where Sally K. Frost was a research associate. Jiro Matsumoto is a professor of biology at Keio University, Yokohama 223 Japan. John D. Taylor is professor and chairman of the Department of Biology, Wayne State University, Detroit, Michigan 48202 and William A. Turner, Jr., serves as his associate. T. T. Tchen is a professor of chemistry at Wayne State University.

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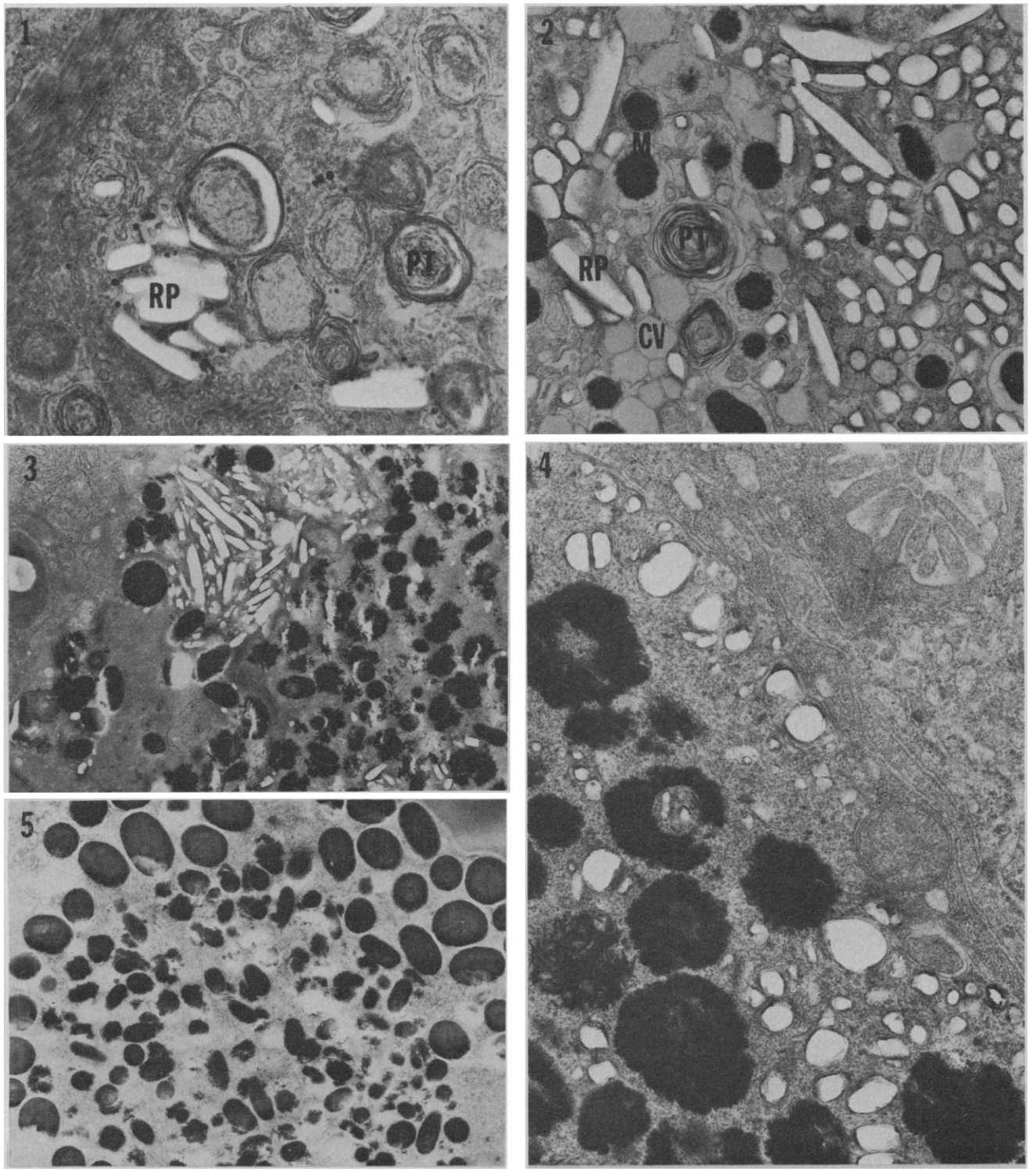


Fig. 1. Erythrophore of the garter snake *Thamnophis proximus*, containing its usual pterinosomes (*PT*) and a few reflecting platelets (*RP*) (× 24,000). Fig. 2. Mosaic chromatophore in the dermis of a *Pachymedusa dacnicolor* larva induced to transform by the local implantation of a thyroxine-cholesterol pellet. Melanosomes (*M*), reflecting platelets (*RP*), pterinosomes (*PT*), and carotenoid vesicles (*CV*) are all present in the same chromatophore (×21,200). Fig. 3. Mosaic chromatophore from the kidney of a *Pachymedusa dacnicolor* froglet containing both developing dermal melanosomes and reflecting platelets (×12,500). Fig. 4. Mosaic chromatophore from the liver of an adult *Pachymedusa dacnicolor*, containing both mature melanosomes and reflecting platelets. The kernel of one melanosome contains unusual inclusions that seem to be small reflecting platelets (×35,400). Fig. 5. Mosaic chromatophore from the kidney of a *Pachymedusa dacnicolor* froglet containing both the smaller developing melanosomes typical of the dermis and the larger melanosomes typical of epidermal melanophores (×12,500).

assumed that prospective pigment cells that migrate to various places in the periphery are not melanoblasts, iridoblasts, or xanthoblasts, but are undetermined cells that are free to differentiate into the chromatophore type or types appropriate for that periphery. Perhaps it is premature to assign a too rigid definition to the stem cell; but, for the purpose of this article, we are referring to a cell present in the neural crest that has the developmental potential to become any of the various pigment cell types. We assume that the stem cell has different developmental capacities from those of other cells of the neural crest, which may give rise to the array of aforementioned derivatives. Exactly when the chromatophore stem cell becomes specifically determined is open to speculation; however, we suspect that, much like the neuroblasts of the autonomic nervous system, the specific phenotype is not established until the site of localization is reached (*1*). The nature of the developmental cues leading to specific chromatophore expression is still unknown.

Mosaic Pigment Cells

The original hypothesis about a common origin of all pigment cells was derived from the discovery of mosaic chromatophores that possess not only their own pigmentary organelles, but also those appropriate to other pigment cells. Well-documented examples of mixed pigment cells include both pterinosomes and melanosomes in erythrophores and melanophores (*5*), and both melanosomes and reflecting platelets in melanophores (*6*) and iridophores (*7*). These iridophores are particularly interesting because those from the iris of a dove contain reflecting platelets that seem to be partially melanized. In other words, two entirely different pigments were found within the same organelle. A similar observation was made on the tapetum lucidum of the teleost *Dasyatis sabina*, where both melanosomes and reflecting platelets occur within the same limiting membrane (*8*). Further refinement and elaboration of the hypothesis awaited new and additional observations, because the possibility existed that the above-mentioned examples were anomalies and not reflective of any general principle. New discoveries have been forthcoming and are presented here in support of a more definitive hypothesis for the common origin of pigment cells.

Of fundamental importance is the fact that, among the mosaic or mixed pigment cells, examples have been found of individual chromatophores that contain three or more different pigmentary organelles. We first observed the presence of melanosomes, reflecting platelets, and pterinosomes in erythrophores of the central red stripe area of the garter snake *Thamnophis proximus*. While such cells were not uncommon, erythrophores containing reflecting platelets (Fig. 1) were more prevalent. The most striking examples of chromatophore mosaicism were observed in the dermis of metamorphosing stages of the leaf frog *Pachymedusa (Agalychnis) dacnicolor*. Melanophores in adults of this species possess an unusually large compound melanosome made up of a eumelanin core surrounded by a fibrous mass (*9*). We now know that this fibrous material is actually a pteridine pigment, pterorhodin (*10*). The adult melanosome forms at metamorphosis from the transformation of the larval dermal melanosome by a deposition of pterorhodin fibers on its surface (*11*).

In experiments involving the premature induction of this transformation, local metamorphic changes were induced in the skin by the subcutaneous implantation of pellets consisting of thyroxine and cholesterol. Electron microscopic examination of the treated skin revealed the presence of mosaic pigment cells. The most striking example (Fig. 2) was a mosaic chromatophore that contained transforming larval melanosomes, reflecting platelets, pterinosomes, and carotenoid vesicles. The last-mentioned were typical of those smooth carotenoid-containing vesicles normally found interspersed between pterinosomes in xanthophores of this and many other species. Obviously, this is an anomalous chromatophore since such an extreme case of mosaicism was never observed in studies of the normal sequence of chromatophore differentiation in this species (*3*). Nevertheless, it manifests aspects of normalcy in that the various organelles it contains are typical of those found in definitive chromatophores even to the extent that various stages of reflecting platelet organellogenesis were observed.

Chromatophore mosaicism is not restricted to the integument. In *Pachymedusa*, chromatophores are found within and on the surface of the liver, kidney, and other organs. In examining chromatophores of the kidney and liver of adult *P. dacnicolor*, we unexpectedly found mosaic chromatophores containing melanosomes, reflecting platelets, and pterinosomes (Fig. 3). In some liver melanophores, the kernel of the adult type melanosome contains an amorphous material, in which seem to be embedded small reflecting platelets (Fig. 4). This is particularly striking because it appears that elements of three different pigmentary organelles are found together within the limiting membrane of one organelle. The presence of large round melanosomes that are clearly characteristic of epidermal melanophores (Fig. 5) was unexpected. In the course of its development the integument of the leaf frog can contain three distinctive melanosomes; of these, two are restricted to the dermis in a precise ontogenetic sequence, and one is a characteristic of the epidermis alone (*11*). In view of these constraints, it is difficult to comprehend the existence of liver or kidney pigment cells possessing simultaneously all three types of melanosomes. Evidently, when the melanophore differentiates in this milieu it is either exposed to different "developmental cues" or is isolated from integumental inhibiting factors that normally restrict the path of melanosomal organellogenesis.

Model for Pigment Cell Differentiation

The discovery of many new and more striking examples of chromatophore mosaicism has strengthened the original hypothesis of a common origin of pigment cells. It now seems that the endoplasmic reticulum provides the structural component in the genesis of pigmentary organelles of all vertebrate chromatophores, and, as such, the endoplasmic reticulum is a candidate for the primordial organelle (*3*). It is appropriate, therefore, to refine the hypothesis in the form of a model (Fig. 6) that will accommodate what is known about the differentiation of the various pigmentary organelles.

The focal point of this scheme is a primordial organelle which can, in response to appropriate developmental cues, give rise to melanosomes, reflecting platelets, or pterinosomes. This preorganelle may be a vesicle formed from the rough endoplasmic reticulum (RER), and may represent an early structural component in the genesis of each pigmentary organelle. That the endoplasmic reticulum has a role in this scheme is probable because it has been implicated in the genesis of all of these organelles (*3*).

Most of our knowledge about chromatophore organellogenesis pertains to melanophores. The melanosomes of homiotherms have been reputed to have formed from a series of fibrillar premelanosomes that were derived from vesicles pinched off of the Golgi complex (*12*). The vesicles contain tyrosinase;

thus, both structural and enzymatic components of the melanosome were considered to be derived ultimately from the Golgi complex. There is now reason to believe that these two components have separate origins (*13*). The structural component of the melanosome is thought to have been formed from a vesicle stemming from the endoplasmic reticulum, with tyrosinase being transported to it in small vesicles that pinch off of the Golgi complex. By their fusion, these two components give rise ultimately to the fully formed eumelanin-containing melanosome typical of both warm- and cold-blooded vertebrates. Probably the best support for this concept comes from the observation that melanosomes of goldfish are not derived from a classical premelanosome, but from a multivesicular body of dual origin (*14*). In this case the outer membrane is derived from the endoplasmic reticulum and the inner vesicles from the Golgi complex. The inner vesicles contain tyrosinase and provide enough enzyme for the ultimate melanization of the multivesicular body. Thus, in the genesis of this melanosome, the fibrillar premelanosome is replaced by a multivesicular body. In both cases, mature melanosomes of similar form and composition are produced. The role of the fibrillar network is not known, but possibly it provides a framework that preserves the ellipsoidal shape typical of such melanosomes. The mechanism of fusion and internalization of the tyrosinase-bearing vesicles in the goldfish melanosomes involves a step in which they are inverted so that finally the enzyme is found on the outer surface of the internal vesicle. Whether this occurs in the formation of other melanosomes is not known. Nevertheless, the important point is that the fusion of a Golgi-derived tyrosinase-containing vesicle with a vesicle from the endoplasmic reticulum seems to be a general mechanism in the genesis of eumelanin-containing melanosomes.

Multivesicular bodies have been implicated also in the formation of phaeomelanosomes. These organelles contain the yellow or red cysteine-containing pigment, phaeomelanin, whose initial steps in biosynthesis are catalyzed by tyrosinase (*15*). Studies on the formation of phaeomelanosomes (*16*) support the view that the endoplasmic reticulum gives rise to the outside of the multivesicular body, and the Golgi complex produces the internal vesicles that contain the tyrosinase necessary to initiate phaeomelanogenesis. Thus, the genesis of both phaeomelanosomes and eumelanosomes is common, and this fact could provide the cellular basis for the sequential formation of these organelles within the same melanocyte, as is seen in agouti mice. Probably, in yellow mice which contain phaeomelanin and in which eumelanogenesis is induced by the administration of melanocyte stimulating hormone (MSH) (*17*), a similar process is involved.

Before proceeding further with the genesis of pterinosomes and reflecting platelets, we should clarify the formation of the "primordial vesicle." Our model implies that this vesicle is of dual origin, stemming either from the stacked RER or directly from endoplasmic reticular blebs pinched off of the outer nuclear envelope. It has been observed that the administration of MSH to leaf frogs results in a profound blebbing of the outer nuclear envelope of melanophores, leading to the pinching off of vesicles and to an increase in RER (*18*). Because there is both an increase in dilated RER and the appearance of immature pigment granules subsequent to the onset of blebbing, it seems likely that these organelles are formed from the blebs. Whether there is a similar relationship between blebbing and RER in the genesis of melanosomes in other melanophore systems has not been investigated.

Vesicles derived from cisternae of the RER or from blebbing of the outer nuclear envelope also seem to be the source of both pterinosomes and reflecting platelets. As yet, there has not been a definitive study of the origins of either of these organelles, but the subject has been touched on in the course of other investigations. An ontogenetic sequence of pterinosomes has been described for *Rana japonica* and, while the exact origin of early pterinosome vesicles from endoplasmic reticulum was not followed, this possibility was strongly inferred (*19*). Such a view was supported by an investigation on leaf frog xanthophore differentiation (*3*), in which it was further observed that in xanthoblasts a series of RER vesicles extended into the cytoplasm as a series of blebs from the outer nuclear envelope. In any event, there seems to be agreement that pterinosomes originate from the RER in the form of a vesicle that may be the "primordial vesicle." These early pterinosomes contain an amorphous material that gradually condenses into a series of internal concentric lamellae typical of the fully formed organelle. How the pteridine pigments get into the pterinosome or where they are localized within the organelle is unknown. It is possible

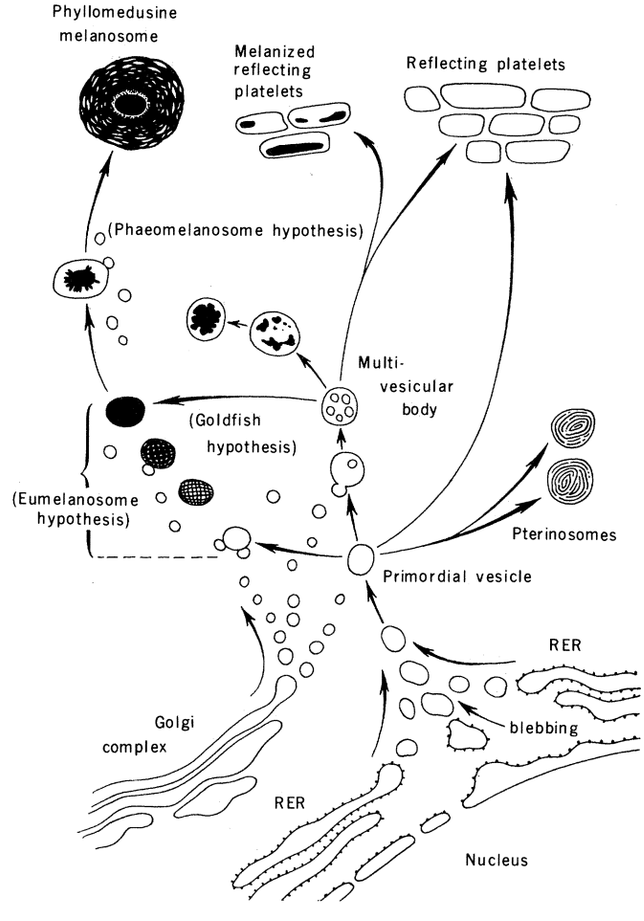


Fig. 6. Hypothetical scheme demonstrating the common origin of pigment cells from a primordial organelle derived from the endoplasmic reticulum.

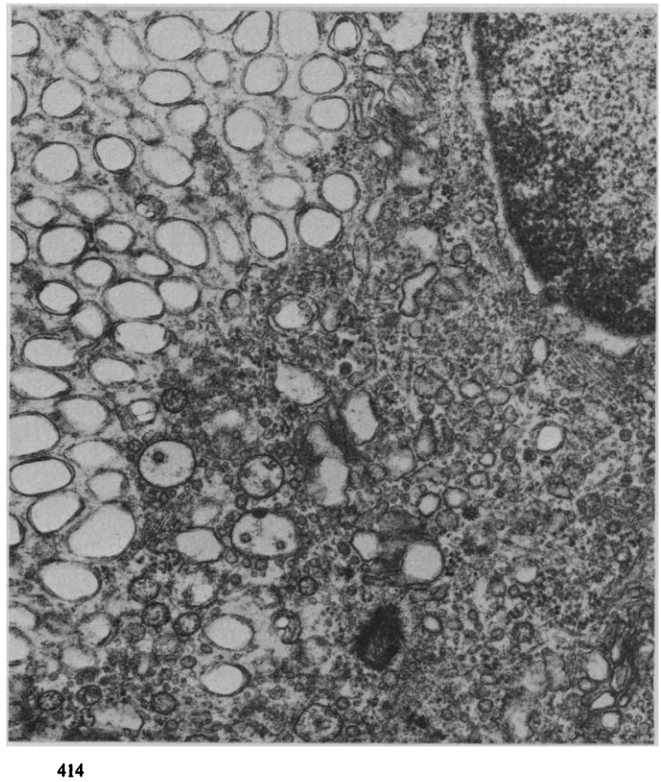
that they are synthesized in situ and that substrates are brought to and incorporated in the early pterinosomal vesicle, or, it may be that finished pteridines are brought to the organelle from other parts of the cell.

Although it was once thought that iridophore reflecting platelets are derived from the Golgi complex, recent evidence indicates that they are derived from the endoplasmic reticulum (*3*). It appears that the orderly array of reflecting platelets within iridophores stems from stacks of endoplasmic reticulum that seem to be layed down as rows of tubules. The tubules gradually assume more rectangular profiles, and purine pigments are deposited in these spaces or cisternae. An alternative center of reflecting platelet generation is evident near the nucleus in older iridoblasts. Here, new platelets appear to be formed from obvious blebs of the outer nuclear membrane. Many of the vesicles that seem to be formed in this way contain internal vesicles and appear to be true multivesicular bodies (Fig. 7). These vesicles radiate away from the nucleus and those that are outermost begin to take on the form of mature reflecting platelets. It seems then, that just as with melanosomes, both the endoplasmic reticulum and multivesicular bodies are implicated in reflecting platelet genesis.

The major thrust of the model that we are discussing is that an endoplasmic reticulum–derived vesicle provides the structural framework for all the pigmentary granules. In a sense, this vesicle serves as a receptacle that receives the various elements necessary for appropriate pigment synthesis. This scheme serves to explain not only normal organellogenesis, but also that of mosaic organelles. For example, the melanized reflecting platelets of the dove iris (*7*) possibly are formed by the incorporation of tyrosinase-bearing vesicles from the Golgi complex into the definitive vesicle undergoing reflecting platelet organellogenesis. Thus, in addition to its incorporation of purine precursors, the developing reflecting platelet may also take in some tyrosinase, leading to the formation of pockets of melanin. Why these iridoblasts should be producing tyrosinase at this time is unknown, but it has been suggested (*4*) that these particular iridophores, which are adjacent to the pigmented epithelium of the iris, are exposed to "developmental cues" for both purine and melanin synthesis. If this is the case, the cells would be expected to synthesize tyrosinase. The notion that melanization of the iridophores of the dove iris is occurring in this manner is suggested by the presence of both electron-opaque vesicles within these mosaic organelles and many multivesicular bodies (Fig. 8).

This model also serves to explain the formation of the unusual melanosomes that are found in the dermal melanophores of adult leaf frogs (Phyllomedusinae). This melanosome is also a pigmentary mosaic containing eumelanin internal to a concentric deposition of a pteridine dimer (*9*). If the limiting membrane of this melanosome is considered to be like that of the original primordial organelle, then the addition, at metamorphic climax, of materials involved in the deposition of the pteridine dimer could be interpreted in the same light as those events that take place in the formation of pterinosomes or of the other pigmentary organelles.

Our model describing pigment cell differentiation not only serves to explain the existence of the various pigment cell mosaics previously described or presented for the first time in this article, but it allows for an understanding of some other hitherto unexplained facts. One of these concerns the transformation of chromatophores in clonal cell culture (*20*). In such cultures of larval bullfrog xanthophores or iridophores, pterinosomes and reflecting platelets, respectively, gradually disappear from these proliferating cells and are replaced by melanosomes. For this transformation to occur, these cells either contain tyrosinase, intrinsically, or can be called upon to produce it quickly. The demonstration of the presence of tyrosinase in frog pterinosomes supports the former, and it is likely that such cells continue to produce the enzyme. Perhaps the production of tyrosinase is a fundamental characteristic of neural crest cells and that this



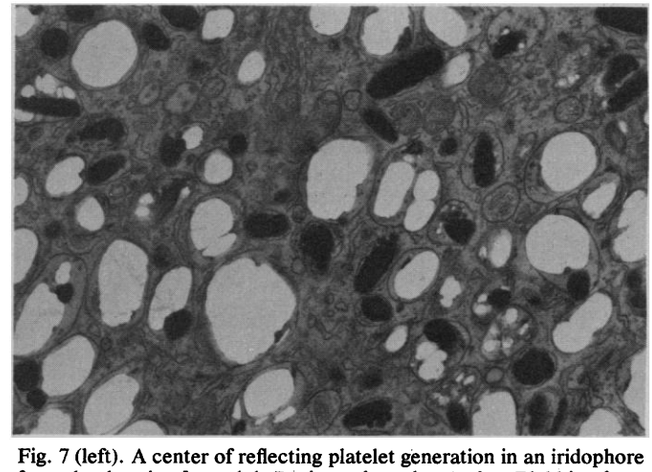


Fig. 7 (left). A center of reflecting platelet generation in an iridophore from the dermis of an adult *Pachymedusa dacnicolor*. Blebbing from the outer nuclear envelope appears to give rise to smaller vesicles. These may develop into obvious multivesicular bodies which, in turn, may become the reflecting platelets which appear to be radiating away from the generation center (×24,000). Fig. 8 (right). Mosaic chromatophore from the iris of the Mexican ground dove, *Columbigallina passerina*, containing mixed organelles in which can be seen what appear to be both reflecting platelets and melanin. Among the mass of mixed organelles are multivesicular bodies. Some of the pigmentary organelles contain small electron dense inclusions which may represent recently incorporated tyrosinase containing vesicles from the Golgi (×20,160).

propensity is expressed unless it is somehow suppressed. This could explain why, under culture conditions, melanophore differentiation occurs so commonly. For example, it takes place from neural crest elements which normally form ganglia (*21*). On the other hand, even melanophores can be transformed, for it has been shown that proliferating bullfrog melanophores in clonal culture will lose melanosomes and form reflecting platelets when they are cultured in a medium containing a high guanosine content (*22*). Possibly, the presence of this augmented level of purine precursor competed successfully with elements for melanogenesis allowing for reflecting platelet expression. The plasticity of pigment cell differentiation in these cases could be explained by the model in terms of what precursors are made available to the "primordial organelle." The prevalence of one type of precursor could be interpreted as the cause for the particular type of organellogenesis that ensues.

A similar lack of specific substrates thus many dictate the course of differentiation. The frequent cases of unexpected melanophore differentiation may be the result of a permissive action stemming from the lack of appropriate precursors. Support for this concept comes from the observation that phenocopies of the melanoid mutant of the axolotl can be produced by the inhibition of xanthine dehydrogenase, which catalyzes the synthesis of certain purines and pteridine monomers that are pigmentary constituents of iridophores and xanthophores, respectively (*23*). Genetic melanoid individuals are characterized by a lack of xanthophores and iridophores and by an increase in melanophore number. These cytological characteristics are duplicated in experimentally produced phenocopies. Thus, it seems reasonable that primordial organelles destined to form pterinosomes and reflecting platelets do not do so because of the lack of purine and pteridine precursors. Instead, they permissively differentiate into melanosomes, with a subsequent increase in melanophore number.

Pigment cells have much in common and, while they are functionally and morphologically distinct from one another, they are rather closely related. This concept can be extended to nonpigment cell differentiation of the neural crest. For example, an expression of chromaffin cell differentiation is the synthesis of the catecholamines epinephrine and norepinephrine. Initial steps in the biosynthesis of these neurohormones involves the formation of dopa (*24*). Since dopa is the key precursor in melanin synthesis by melanophores, another common feature of various neural crest derivatives is revealed. Furthermore, the initial step is catalyzed by phenylalanine hydroxylase, which requires a common pteridine as a cofactor. Thus, still another neural crest derivative, the xanthophore, seems to be related to chromaffin cells. Observations of these types suggest that the origin of the individual neural crest derivatives is rather close and that by the use of similar substrates for diverse types of differentiation, a diversity of differentiation from common stem cells was achieved.

Our hypothetical model presented to explain chromatophore differentiation is still only a working model. It represents a reasonable explanation of the existing data about chromatophore development. The model puts these data in a perspective that should stimulate continued experimentation to provide answers to many questions, dealing with the nature of the "developmental cues" that presumably call forth the expression of a given type of pigment; and whether specific activation or repression (*25*) of chromatophore genes is involved. While we know something about the intrachromatophore synthesis of the melanins, little is known about the cellular synthesis of purines and pteridines. Since these two classes of pigments are related, still unknown is how the synthesis of one affects the other. Of great importance is the nature of the internal membranes. What allows the Golgi-derived vesicles to fuse with the endoplasmic reticulum? What characteristics of the "primordial organelle" allow for the selective passage of substrates involved in pigment synthesis or deposition? Many of them cannot be answered at present because of the lack of appropriate techniques. The stimulus provided by this hypothetical scheme should lead to experimentation that will provide knowledge for a better understanding of not only chromatophore differentiation, but of all neural crest derivatives as well.

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24. It should be pointed out that while chromaffin cells synthesize dopa they do so through the enzyme tyrosine hydroxylase, which cannot further mediate the oxidation of dopa. In contrast, melanophore systems utilize tyrosinase in the sequential oxidation of tyrosine to dopa and of dopa to dopaquinone. These facts do not weaken the argument that we are developing about a key position for the substrate dopa. In any case, there may have been evolutionary changes involved in the enzymes concerned with dopa metabolism such that tyrosinase is really a double entity made up of both tyrosine hydroxylase and dopa oxidase as is discussed by K. Jimbow, W. Quenedo, T. B. Fitzpatrick, G. Szabo [*J. Invest. Dermatol.* **67**, 72 (1976)] and K. Nishioka [*Eur. J. Biochem.* **85**, 137 (1978)].
25. A. I. Caplan and C. P. Ordahl [*Science* **201**, 120 (1978)] have proposed recently a model involving progressive gene repression as a mechanism for controlling differentiation. Such would not be inconsistent with observations made on pigment cell development.

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