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Research Article

STUDY OF PLASTICITY AND REPROGRAMMING ABILITY OF SOMATIC CELLS UNDER THE INFLUENCE OF VITAMIN A

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ABSTRACT
Purpose Study of terminally differentiated ocular and tail tissue changing the fate under the influence of vitamin A. Present study have shown that differentiated cell types may loose their definitive characteristics and acquire features of another specialized cell type. Young (3 toe stage) and mature (5 toe stage) tadpoles of the frog, *Euphylictis cyanophlyctis* were employed as experimental animals. Experiments completed in two phases: in the first part of experiment lenses were extracted from right eye balls of tadpoles and treated with vitamin A; in the second part of the experiment meshed lentectomized eye ball tissues implanted into the pit made on mid lateral position of the tail of young and mature tadpoles and were treated with vitamin A. The results obtained in the present study give clear evidence of plasticity and reprogramming of terminally differentiated ocular and tail tissue into limb, lens, retina and even complete eye under the influence of vitamin A. From the results obtained it can be concluded that even differentiated cells might be reprogrammed, by exposing to a new environment and become another cell type. Vitamin A is found to be good model for homeotic transformation of corneal tissue to lens, ocular plus tail tissue to limb, lens, retina and even eye at ectopic site (mid lateral position of the tail) in anuran frog tadpoles.

Key Words: *Vitamin A, Ocular- Tail Tissue, Reprogramming, Limb, Lens, Eye*

INTRODUCTION
Plastic behavior of cells is generally hallmark of embryonic development although it is reported that adult mesenchymal stem cells may differentiate in culture into a multitude of mature cell types including epithelial cells. This opens the way to the use of these stem cells for the construction of new tissues and organ for therapeutic purpose.
Ocular tissues are found to be a highly powerful model for studying stability and plasticity in differentiation of tissue cells. In vitro studies have revealed that dormant potential of pigmented epithelial cells (PECs) to transdifferentiate into lens cells is widely conserved throughout vertebrate species including aged humans (Kodama and Eguchi, 1995; Tsonis *et al.,* 2001; Jangir *et al.,* 2005; Sharma *et al.,* 2010) .It was found that differentiated cells of the corneal epithelium are converted to hair, along with their associated stem cells then interfollicular epidermis by means of a multistep process triggered by dermal developmental signals (Pearton *et al.,* 2004). Ferraris *et al.,* (2000) also reported that adult central corneal epithelium, which is comprised of differentiated cells and contains no stem cells, can be reprogrammed become hairs and interfollicular epidermis under the influence of an embryonic hair forming dermis. This means that committed transient amplifying differentiating cells are able to transdifferentiate into cells of another ectodermal lineage. In anuran amphibians like *Xenopus laevis* lentectomy may be

followed by regeneration of a new lens from the cornea. This regeneration occurs via transdifferentiation of the outer corneal epithelium (Filoni, 1982; Bosco, 1988). In urodele amphibian like newt when lens is removed, regeneration occurs by the dedifferentiation of the pigmented epithelial cells (PECs) of the dorsal iris (Tsonis, 1999, 2000). This gives clear evidence of plasticity and reprogrammed fate of terminally differentiated tissue.

Vitamin A and its derivative, retinoid have remarkable effect on different systems in developing embryos. Eyes are the organ whose development is largely dependent on retinoid and their receptors. The most remarkable of all the effects of retinoid on embryonic system is the homeotic transformation of tail into leg (Mohanty-Hejmadi *et al.,* 1992; Maden, 1993; Mahapatra and Mohanty-Hejmadi, 1994). In present paper we report that the meshed ocular explants implanted in the pit made on mid lateral position of the tail of vitamin A treated tadpoles transformed into limb, lens, retina and even complete eye.

MATERIALS AND METHODS

Tadpoles of the frog, *Euphlyctis cyanophlyctis* were used as experimental animals. Tadpoles were reared from eggs collected from ponds. Two developmental stages viz 3 toe stage (young) and 5 toe stage (mature) tadpoles were employed for experiments. Tadpoles were anaesthetized with 1: 2000 MS222. For control group, tadpoles were reared in conditioned tap water. While tadpoles for experimental groups were exposed to 15 IU/ml vitamin A for 3 days and then transferred to conditioned tap water.

Plan of experiment (Table 1)

The experiment was designed into two Series I & II.

Series	Developmental stage of tadpoles employed	Group	Day of preservation	Number of operated animals
SI Tadpoles with lentectomized right eye	3 toe stage young tadpoles	Control S IA	3 7 20	5 5 10
		Vitamin A treated S IB	3 7 20	5 5 10
	5 toe stage mature tadpoles	Control S IC	3 7 20	5 5 10
		Vitamin A treated S ID	3 7 20	5 5 10
SII Tadpoles bearing meshed ocular tissue explants in their tail	3 toe stage young tadpoles	Control S IIA	3 7 20	5 5 10
		Vitamin A treated S IIB	3 7 20	5 5 10
	5 toe stage mature tadpoles	Control S IIC	3 7 20	5 5 10
		Vitamin A treated S IID	3 7 20	5 5 10

Table 1: Series I consists of those tadpoles which were subjected to operate their lenses from right eye and reared in 15IU/ml vitamin A solution (treated). Whereas series II comprises host tadpoles with meshed ocular tissue (lentectomized eye ball tissue) explants in their tails.

Preparation of explants Meshed lentectomized eye ball of 3 toe stage young tadpoles were used as explants. For this purpose their eye balls were taken out from 5 donor young tadpoles. Lenses were extracted from these eye balls. Lentectomized eye balls were pooled and meshed or subjected to homogenize and thus made explants.

Preparation of recipients Young and mature tadpoles were treated as recipient or host animals. The animals were anaesthetized (1:2000MS222 solution) before operation. A semicircular pit was made on mid lateral position of the tail.

Implantation A small piece of meshed ocular tissue was implanted into the pit on recipient tadpole's tail. After insertion of the explants, skin flap was covered over it. Operated animals with implants were kept immovable for half an hour by keeping them half submerged in MS222 solution. Operated tadpoles of both series were preserved in Bouin's solution on day 3, 7 and 20 for histological evaluation. Experiment was terminated on day 20 after operation.

RESULTS

The results obtained are presented in Table2.The lens regenerative power was found in young as well as mature tadpoles of *Euphlyctis cyanophlyctis* (Table 2 Series I). However, percentage of regeneration declined with the age of animal, it was 35% in 3 toe stage young tadpoles of untreated control group SI-A while 20% in 5 toe stage mature tadpoles of group SI-C (control). Vitamin A was found to accelerate the percentage of lens regeneration it was 60% in young (SI-B) and 50% in mature tadpoles (SI-D). Thus the declining trend of lens regeneration with the age of animal was similar to that of untreated control group animals.

It is very interesting to note that regeneration of lens was found to occur from inner layer of outer cornea. Particularly in those tadpoles which were treated with vitamin A and whose iris epithelium get remain touched with cornea during lentectomy; lens regenerated from outer corneal epithelium. Histological observations revealed that in such cases where iris kept persistent connection with cornea, lens regenerates from the inner layer of outer cornea (Figs.1&2). The stages of lens regeneration from the cornea are almost similar to the ones seen from the dorsal iris. A vesicle is first formed and then gradually lens fibers accumulate. The percentage of lens regeneration from cornea was found higher in vitamin A treated tadpoles. (See Table 2).

The results obtained from tadpoles of series II are unique. The result shows plasticity of well differentiated ocular and tail tissues under the influence of vitamin A. The findings are presented in the Table 1 suggest that vitamin A changes the fate of ocular tissue explants at ectopic site (mid lateral region of tail) and results give clear evidence of transdifferentiation / homeotic transformation of terminally differentiated somatic cells. The results as shown in the table also reveal that even somatic cells can reprogrammed and differentiate into different cell line under experimental condition. In five out of twenty young host tadpoles complete hind limbs with pelvic girdle developed at the site of implantation (mid lateral region of the tail) (Figs.3,4&5). Even two limbs have also been reported in two out of these five host vitamin A treated tadpoles. (Fig.5) This type of transformation (from ocular + tail tissue into limb) was also reported in mature host tadpoles but percentage was low (Table2).

Table 2: Plasticity and reprogramming of differentiated ocular and tail tissue of tadpoles under the influence of vitamin A

Series	Development-al stage of tadpoles employed	Group	Day of prese-rvation	Number of animals preserved	Number(%)of structure developed				
					Lens	Retina	Whole eye	Limb	Unidentif-ied structure
SI	3 toe stage young tadpole	SI-A	3	5	-	-	-	-	-
		Control	7	5	2	-	-	-	-
			20	10	5 (35%)	-	-	-	-
Tadpoles with lentectom-ized right eye		S I-B	3	5	-	-	-	-	-
		Vitamin A treated	7	5	4	-	-	-	-
			20	10	8 (60%)	-	-	-	-
	5 toe stage mature tadpoles	S I-C	3	5	-	-	-	-	-
		Control	7	5	-	-	-	-	-
			20	10	4 (20%)	-	-	-	-
		S I-D	3	5	-	-	-	-	-
		Vitamin A treated	7	5	2	-	-	-	-
			20	10	8 (50%)	-	-	-	-
SII	3 toe stage young tadpole		3	5	-	-	-	-	5
		S II-E	7	5	-	-	-	-	5
		Control	20	10	2 (10%)	-	-	-	8 (90%)
Tadpoles bearing meshed ocular tissue explants in their tail.			3	5	-	-	-	-	5
		S II-F	7	5	2	1	1	1	0
		Vitamin A treated	20	10	2 (20%)	2 (15%)	2 (15%)	4 (25%)	0 (25%)
	5 toe stage mature tadpoles			5	-	-	-	-	5
		S II-G	3	5	-	-	-	-	5
		Control	7	10	2	-	-	-	8
			20		(10%)				(90%)
		S II-H	3	5	-	-	-	-	5
		Vitamin A treated	7	5	2	2	-	-	4
			20	10	2 (20%)	- (10%)	2 (10%)	3 (15%)	0 (45%)

Histological study clearly shows the development of skeletal structure of pelvic girdle and hind limb in vitamin A treated tadpoles at grafted site of the tail. The developmental stages of ectopic hind limbs (EHLs) along with pelvic elements observed at different time intervals were found almost similar to that of embryonic development of limb. A thick apical ectodermal cap (Fig.3) was formed in vitamin A treated tadpoles after 3 days post implantation. Later on it becomes limb bud like structure and then developed and differentiates into ectopic hind limb by day 20 post implantation. The whole pelvic segments as shown in Figs.4&5 with skeletal elements which normally do not occur in the tail are generated. Fig. 5 shows well differentiated two pelvic girdles developed in the host tadpole's tail with respective skeletal segments.

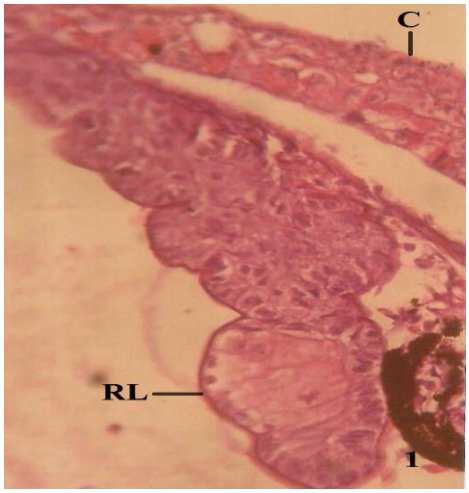

Figure.1: Microphotographs of a section passing through the lentectomized eye of 7day vitamin A treated tadpole. Note lens forming cells originating from the inner layer of outer cornea (100X).

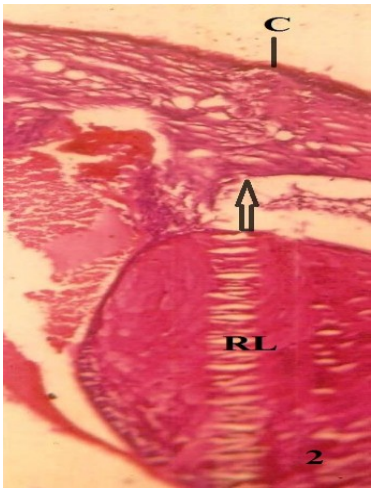

Figure.2: Microphotograph of a section passing through the lentectomized eye of 20 day vitamin A treated tadpole. Note regenerated lens showing connection with cornea (→) (40X). Regenerated lens showing well differentiation of lens fibers.
(→) lens forming cells originating from cornea.

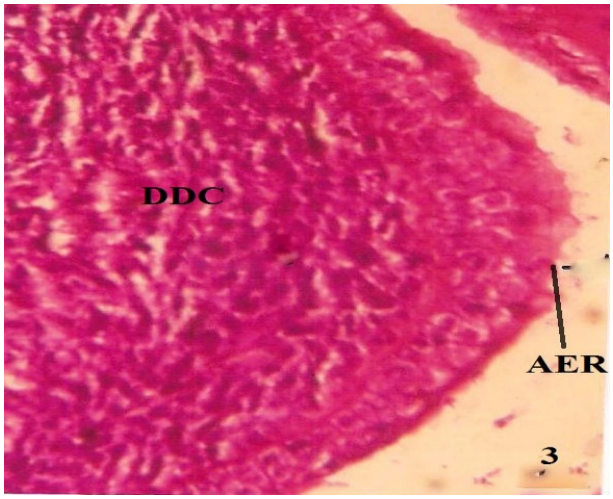

Figure 3: Microphotograph of a section passing through the graft tissue in the tail of 3 day vitamin A treated tadpole. Note homeotic transformation of ocular graft into limb bud like structure. Fig. also showing accumulation of dedifferentiating cells beneath AER (100X).

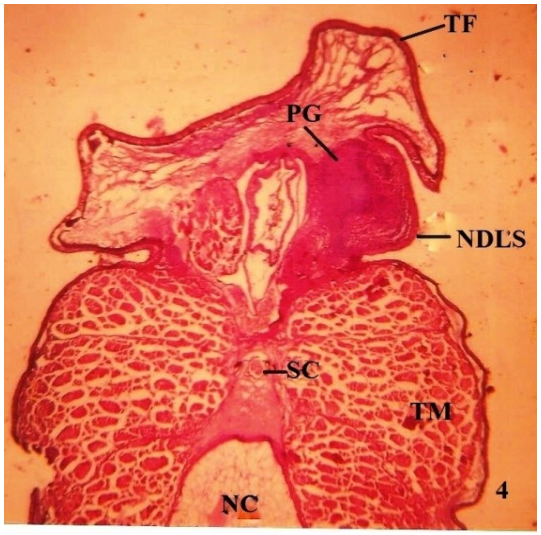

Figure 4: Microphotograph of a section passing through the graft in the tail of 7 day vitamin A treated tadpole. Note homeotic transformation of ocular graft into limb elements with pelvic girdle. (40X)

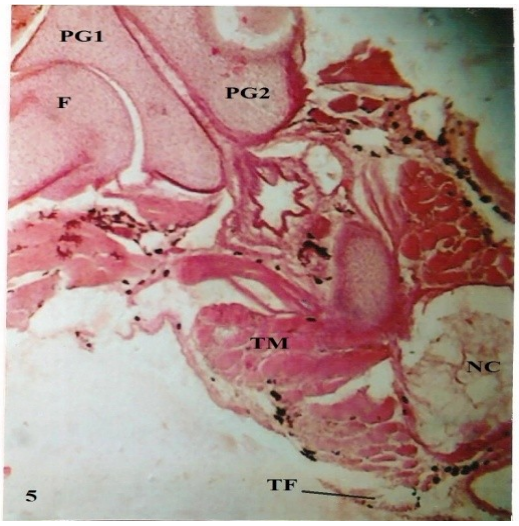

Figure 5: Microphotograph of a section passing through the graft in the tail of 7 day vitamin A treated tadpole. Note two well defined pelvic girdles (PG1 & PG2) developed from ocular explants in the tail. Two hind limb also articulated respectively with the newly developed ectopic pelvic girdle (PG1 & PG2). (40X)

Abbreviations for figure 1-5. *PG = Pelvic girdle: NDL = Newly developed limb: TF = Tail fin: F = Femur: NC = Notochord, NDLS = Newly developed limb bud like structure: SC = Spinal cord: TM = Tail musculature, RL = Regenerated lens: C = Cornea: DDC = Dedifferentiated cells: AER = Apical ectodermal ridge*

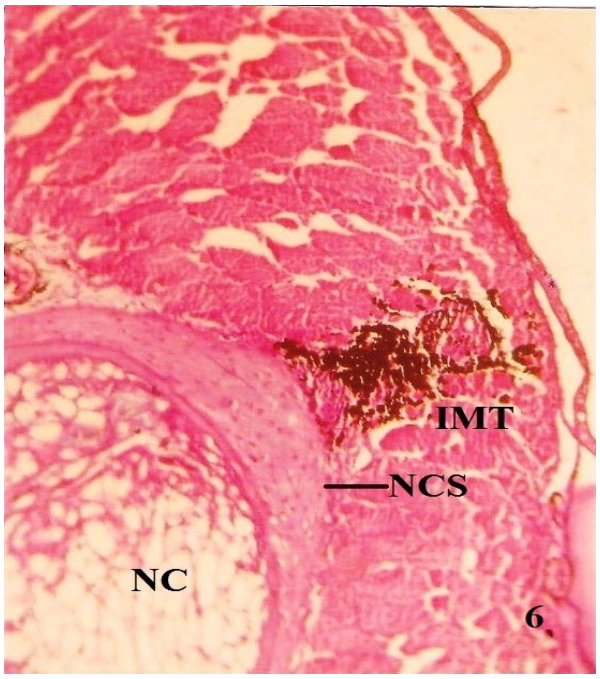

Figure 6: Microphotograph of a section passing through the ocular graft in the tail of 3 day vitamin A treated tadpole. Note close association and involvement of notochordal sheath tissue in the differentiation of explants. Depigmentation (⟶) of pigmented epithelial cells begin. (40X)

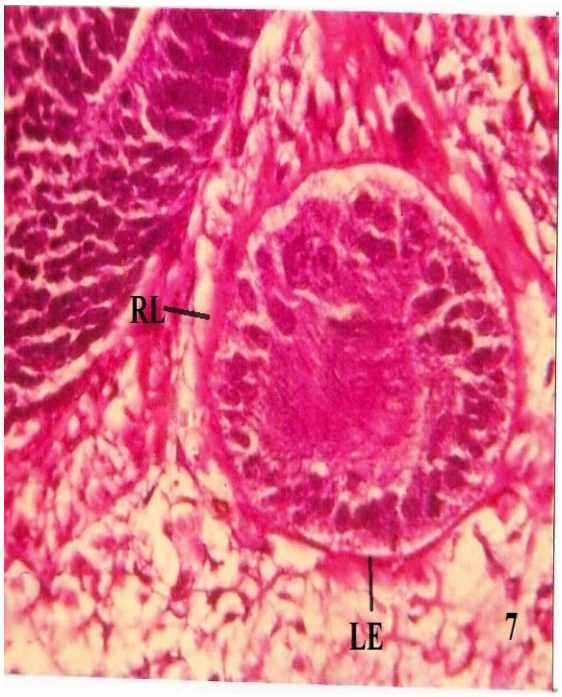

Figure 7: Microphotograph of a section passing through the grafts in the tail of 20 day vitamin A treated tadpole.

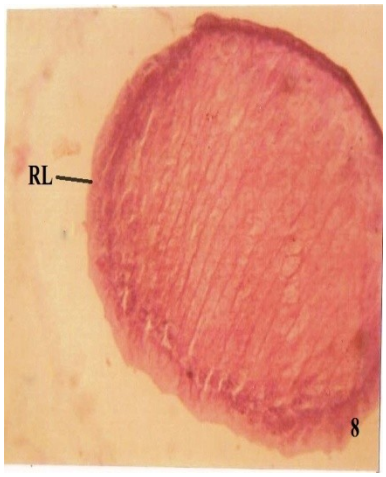

Figure 8: formation of cellular lens (100X). Well differentiated lens is shown in Fig.9 (100X).

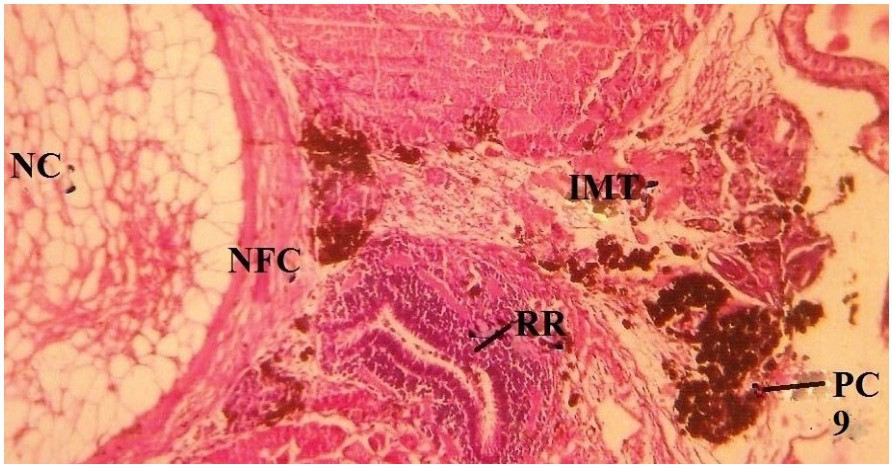

Figure.9: Microphotograph of a section passing through the ocular graft differentiating into retina in the tail of 20 day vitamin A treated tadpole. (40X)

Abbreviations for figure 6, 7, 8. *RL = Regenerated lens: LE = Len epithelium: LV = Lens vesicle: NC = Notochord PC = Pigmented epithelium cells: IMT = Implanted ocular tissue: NS = Notochordal sheath*
Abbreviations for figure 9 *RR = Regenerated retina; PC = Pigmented epithelial cells: NFS = Notochordal fibrous sheath; IMT = Implanted tissue; NC = Notochord*

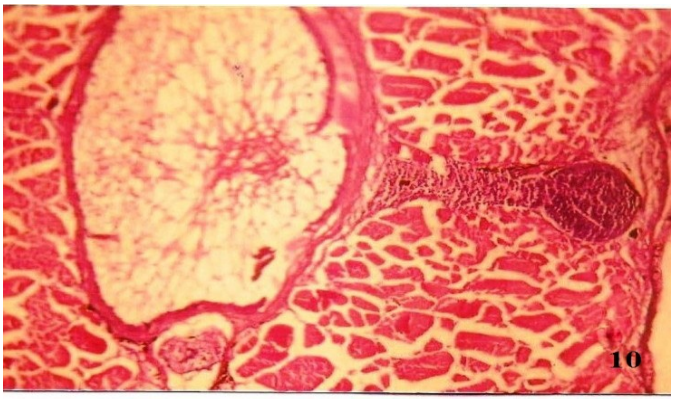

Figure 10: Microphotograph of a section passing through the newly developed complete eye from the ocular graft in the tail of 20 day vitamin A treated tadpole. Note eye like structure transdifferentiated from the graft linked with notochordal sheath by a stalk like structure. (40X)

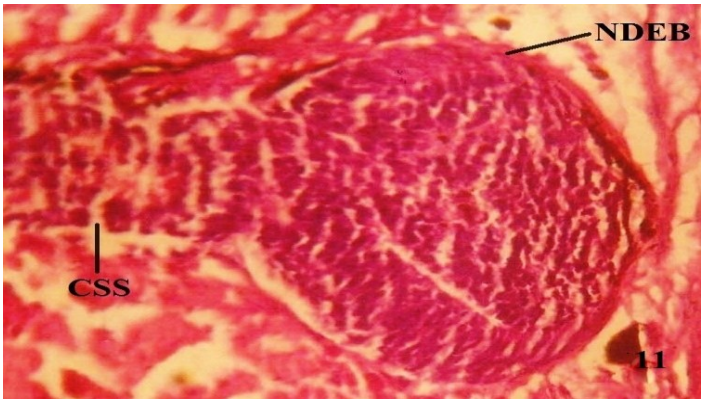

Figure 11: shows the magnified view of figure 8. (100X)

Abbreviations for figure 10 & 11. *CSS = Cellular stalk like structure: NDEB = Newly developed eye ball.*

In other cases of vitamin A treated groups (SII E-H) formation of lens, retina and even complete eye have been reported. The percentage of lens, retina and complete eye formation declined with the age of animal; it was 20%, 15%and 15% in young tadpoles and 20%, 10% and 10% in mature 5 toe stage tadpoles respectively. Except lens no other ectopic structure like retina, eye and limbs were developed from explants in untreated control animals. In majority of operated tadpoles the grafts or explants could not further differentiate and ultimately disappeared by day 20 post implantation or remain as unidentified structure. Histological observations revealed that by 3rd day of implantation cell-cell contact of meshed extract were loose. Many blood cells not only gathered around the reaggregate, but also invaded the clefts between cells. Depigmentation starts by 5th – 7th day of implantation within the reaggregate (Fig.6). Depigmented cells have a tendency to take a flattened tubular or vesicular form. Later on by day 15 of implantation some cells of reaggregates proliferated to fill the inner space of vesicles (Fig.7). The growth and fiber formation of lens epithelial like cells proceeded in a manner similar to the normal stages of regeneration (Fig.8).

The most spectacular result obtained from the present ectopic transplantation of meshed ocular tissue is the formation of well defined retina and even complete eye in vitamin A treated animals. Although the percentage of these structures was low (Table2). The transdifferentiation of meshed pigmented epithelium into retina involves depigmentation of its cells, their proliferation and then formation of layers in newly formed retina (Fig.9). In few cases of vitamin A treated host tadpoles formation of complete eye was reported. The sequential development of complete eye showed that meshed ocular tissue makes contact with host's notochord. Notochord sheath became bulbus (thick) and induces the explant to transdifferentiate into eye like structure. Newly developed eye showed a connection with notochord sheath by a stalk like structure (Figs.10& 11), probably it could develop as optic nerve. Histological observations give clear evidence of enlargement of the notochord in implanted cases particularly of vitamin A treated animals.

DISCUSSION

The present findings give clear evidence of plasticity of terminally differentiated ocular tissue cells under the influence of vitamin A. Vitamin A was found to be a good model in signaling the initiation of homeotic transformation of meshed ocular tissue into limb, lens, retina, and whole eye. The results also confirm the stage dependent effect of vitamin A on homeotic transformation. As mentioned in the table the percentage of limb formation from the grafts was high in young 3 toe stage tadpoles in comparison to that of mature 5 toe stage tadpoles. Even two limbs have also been reported with respective pelvic girdle in vitamin A treated young tadpoles. Such type of homeotic transformation was also reported by Mohanty –Hejmadi *et al* 1994 & Maden 1993. Mohanty –Hejmadi *et a*l (1994) reported homeotic transformation of tail tissue into limb under the influence of vitamin A. It was hypothesized that homeotic transformation of tail into legs is initiated by activation, inhibition or increase in transcriptional activity of certain genes, some of which are responsible for the respecification of tail cells to leg cells Maden (1993). It is also proposed that the tail blastemas are converted by RA to a state that is synonymous to that of the pre limb bud flank cells. This respecification is then followed by interaction between the blastema and the stump cells to generate hind limbs sites on the tail (Bryant & Gardiner 1992). In the present study of meshed ocular tissue implants might induce tail tissue at grafted site to transdifferentiate into pelvic and hind limb structures. However, it is difficult to determine whether during homeotic transformation the explants tissue is directly

converted to limb or it induces tail tissue first to transformed into flank tissue and then into the limb. Other possibility of homeotic transformation may be that the explants tissue induces notochordal sheath, mesenchymal tissue of the tail and expansion of limb field into the tail which may give rise new limb from the injured tail site. During this transformation, most noteworthy is the enlargement of the notochord in the treated group. Thus there is a strong possibility that the notochordal cells may play a role in induction of the pelvic segment leading to the formation of limb buds and consequently complete limb. Christ *et al* (2000) reviewed that somite formation is not possible without nerve cord and the notochord. In present experimental design of the implantation site in the tail, both nerve cord and notochord are present. Nerves grow out to innervate EHLs. Therefore, it can be speculate that under the influence of vitamin A, notochord mediates the expression of the key Hox genes for the establishment of the pelvic region. The Hox genes are likely to be components of homeotic transformation of the tail into legs (Maden1993; Mohanty- Hejmadi& Crawford 2003). As far as development of ectopic lens, retina and whole eye from the ocular explants is concerned, it is clear evidence of the fact that the eye is not necessarily needed for dedifferentiation and transdifferentiation of meshed ocular tissue cells. Ito *et al* (1999) also reported development of ectopic lens into the blastema of the forelimb in the newt. They constructed lens into the limb by transplanting iris PECs into the limb blastema. But how complete eye developed in vitamin A treated cases from the meshed explants is yet not clear. Vitamin A might accelerated dedifferentiation and proliferation of meshed tissue cells and subsequently complete eye developed. In the present study transdifferentiation of cornea to lens was reported very frequently in lentectomized eyes of vitamin A treated tadpoles. Several workers have also reported that in some anuran amphibians like *Xenopus laevis* lens regenerate from cornea (Freeman1963; Filoni *et al* 1997 & Henry and Elkins 2001). This ability of cornea (transdifferentiation of cornea to lens) may be related to it competence. The larval cornea and lens are originally derived from the same embryonic tissue, and the phenomenon of lens regeneration from cornea may simply represent a condition in which there is persistent competence of embryonic and larval ectoderm to respond to key lens inducing signals.

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REFERENCES

Bosco L (1988). Transdifferentiation of ocular tissue in larval Xenopus laevis. *Differentiation* **39** (1) 4-15.

Bryant S V & Gardiner D M (1992). Retinoic acid, local cell-cell interactions and Pattern formation in vertebrate limbs. *Developmental Biology* **152(1)** 1-25**.**

Christ B, Huang R & Wilting J (2000). The development of the avian vertebratal column. *Anatomical Embryology* **202(3)** 179-194.

Eguchi G & Itoh Y (1982). Regeneration of the lens as a phenomenon of cellular epithelial cells. *Transactions of Ophthalmological Society UK* **102(3)** 380–384.

Eguchi G & Kodama R (1995). From lens regeneration in the newt to *in-vitro* transdifferentiation of vertebrate pigmented epithelial cells. *Semin Cell Biology* **6** 143–149.

Ferraris C, Chevalier & Favier B (2000). Adult corneal epithelium basal cells possess the capacity to activate epidermal, pilosebaceous and sweat gland genetic programs in response to embryonic dermal stimuli. *Development* **127** 5487–5495.

Filoni S, Bosco L & Cioni C (1982). The role of neural retina in lens regeneration from cornea in larval *Xenopus laevis*. *Acta Embryology Morphology & Experimental.* **3** 15–28.

Filoni S, Bernardini S, Cannata SM, D' Alessio A (1997). Lens regeneration in larval Xenopus laevis; Experimental analysis of the decline in the regenerative capacity during development. *Devlopmental Biology* **187(1)** 13-24.

Freeman G (1963). Lens regeneration from the cornea in *Xenopus laevis*.*Journal of Experimental Zoology* **154(1)** 39–66.

Henry J J & Elkins M B (2001). Cornea-lens transdifferentiation in the anuran, *Xenopus tropicalis. Devlopment Genes & Evolution* **211(8-9)** 377-87.

Ito M, Hayashi T, Kuroiwa A & Okamow M (1999). Lens formation by pigmented epithelial cell segregate from dorsal iris implemented into limb blastema in the adult newt. *Dev Growth Differ* **41** 429-440.

Jangir O P, Shekhawat D S, Garg S & Goswami M (1995). A study of lens regeneration in the tadpoles of *Rana cynophylictis* under the influence of vitamin A; (XI National Symp. Developmental Bio. Rohtak) pp 31.

Jangir O P, Suthar P, Shekhawat D V S, Acharya P, Swami K K & Sharma M (2005).The "Third Eye" – A new concept of transdifferentiation of pineal gland into median eye in amphibian tadpoles of *Bufo melanostictus*. *Indian Journal of Experimental Biology* **43** 671-678.

Maden M (1993). The homeotic transformation of tails into limb in *Rana temporaria*. *Devlopmental Biology* **159(2)** 379- 391.

Mahapatra & Mohanty-Hejmadi (1994). Vitamin A-Mediated Homeotic Transformation of Tail to Limbs, Limb Suppression and Abnormal Tail Regeneration in the Indian Jumping Frog *Polypedates maculates*. *Development, Growth & Differentiation* **36** (3) 307–317.

Mohanty-Hejmadi P, Dutta S K & Mahapatra P (1992). Limbs generated at the site of tail amputation in marbled ballon frog after vitamin A treatment. *Nature* (*London*) **355** 352–353.

Mohanty-Hejmadi P & Crawford M (2003). Vitamin A: regeneration and homeotic transformation in anuran. *Proceedings of the* Indian National *Science* Academy **69**(5) 673- 690.

Pearton D J, Ferraris C & Dhouailly (2004). Transdifferentiation of corneal epithelium: evidence for linkage between segregation of epidermal stem cells and the induction of hair follicles during embryogenesis. *International Journal of Devlopmental. Biology* **48** 197-201.

Sharma M, Jangir O P, Jhajharia S, Singh S, Singh V & Nagal A (2010). Transdiffirentiation of iris pigmented epithelial cells of *Euphylyctis cyanophylictis* tadpole into lens *in vitro*. *Indian, Journal of Experimental Biology* **48** 17-25.

Tsonis P A (2000). Regeneration in vertebrates. *Developmental Biology* **221** 273– 284.

Tsonis P A, Trombey M T, Rowland T, Chandraratna R A & Tsonis D R (2000). Role of retinoic acid in lens regeneration. *Developmental Dynamics* **219** 588–593.

Tsonis D R K, Tomarev S I & Tsonis, P A (1999). Regulation of prox-1 during lens regeneration. *Investigative Ophthalmology Visual Science* **40** 2039–2045.

Tsonis P A (2004). Stem cells from differentiated cells. *Molecular Intervention* **4** 81–83