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The "Third Eye"— A new concept of trans-differentiation of pineal gland into median eye in amphibian tadpoles of *Bufo melanostictus*

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Median third eye was found to develop from transplanted pineal gland of external gill stage tadpoles in the recipient 5 toe stage tadpoles of *Bufo melanostictus*. Pineal gland along with a bit part of brain tissue of the donor external gill stage tadpole was cut out and transplanted into a pit made between two lateral eyes of 5 toe stage recipient tadpoles. Half of the operated tadpoles were treated with vitamin A (15 IU/ml.) for 15 days. Median "third eye" was found to develop in the both untreated and vitamin A treated tadpoles. However, vitamin A increased the percentage of the development of median eyes. Morphological and histological study revealed that newly transformed median eyes were similar to that of normal functional eyes. A stalk like structure developed which connects the median eye to the brain. The median third eye could not develop when pineal gland of 5 toe stage mature tadpole was transplanted into the tadpole of the same age.

Keywords: *Bufo melanostictus*, Pineal gland, Trans-differentiation, Third eye, Vitamin A

The term trans-differentiation denotes the changes of the determined state of a cell to an indifferent state, which initiate a new pathway of differentiation. Thus, it is a process by which differentiated cells alter their identity to become some other distinct cell type. It is particularly obvious in segmented animals like insects in which one body segment may be transformed into some other body part. Hadorn[1] and Shearn *et al.*[2] studied the trans-differentiation in imaginal disc cells of *Drosophila melanogastor* and found that the frequency of trans-differentiation is co-related with increase in proliferation of cells. Most of the known trans-differentiation steps were realized by homeotic mutation which causes the partial replacement of a body part by structures normally belonging to some other body part[3-5]. Trans-differentiation is not restricted to the invertebrates only but is widely distributed in the vertebrates also.

Under certain conditions, differentiated cells are not stable and this provides demonstration of potential reversibility of pattern of gene activity. Classic example of such a change is regeneration of lens from pigmented epithelial cells of the iris in the eye[6].

Another example may be de-differentiation in which there is loss of differentiated characteristics of muscle cells in newt limb regeneration and the ability of these cells to give rise to cartilage[7]. A number of cell types have been found to undergo such a change in tissue culture particularly when the culture conditions are altered by the addition of chemical agents[8].

With reference to evolutional transformation in structure and function, the pineal gland is found to be unique and useful model system for developmental neurobiology and cell culture as it offers a useful tool for the understanding of development and cell trans-differentiation[9].

It is a generally accepted view that primitive vertebrates (Ostracoderms and Placoderms) possessed a median third eye apart from the paired lateral eyes. Each being an epiphysial evagination formed as evagination of the diencephalon, wall of the brain in the same fashion as lateral eyes[10]. In the later evolutionary history of vertebrates while the pained lateral eyes gained prominence, assuming the main visual function, the median pineal eye generally degenerated and the pineal foramen was closed. Otherwise in most of the lower vertebrates a median foramen in the skull roof was there to allow the passage of epiphysial evagination. In living forms vestigial median eye persists in some vertebrates like spotted dogfish *Scyliorhinus caniculus*, the salamander *Ambyostoma tigerinum*, the frog *Acris grylus* and the lizard tuatara *Sphenodon punctatum*[11-14]. Whereas, non-visual photoreceptor

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pineal organ is present in most of the vertebrates including man. Sato[15] reported that avian pineal organ represents a transitional type between a photosensory organ of lower vertebrates and the endocrine gland of mammals and shows remarkable changes in its innervation and structure during ontogeny. Immunoreactivity for photopigments is reduced during the post hatching development of chicken whereas neuron specific enolase (NSE = cytoplasmic marker of neurons and neuroendocrine tissue) in the immunoreactive pinealocytes increases remarkably in number in the end vesicle of the domestic fowl with age, followed by a gradual expansion towards the proximal portion (secretary part). Thus, it reflects the sequence of changes leading from pineal sense organs to pineal gland. The pineal of adult birds is a photo-endocrine organ and is considered to synthesize and secrete melatonin in intrapineal rhythm which can be modified by direct light stimulation of the pineal photo receptor, whereas the pineal gland in mammals is an endocrine organ and generally does not exhibit neuronal characteristics. However, it is known that under culture conditions cells from newborn rat pineal express properties characteristic of photoreceptors[16].

Evolutional transformation characteristics of pineal gland and previous findings in which median eye was found to develop from the pineal gland *in situ* after removal of both lateral eyes in the early gill stage tadpoles of *Bufo melanostictus*[17,18] motivated the present study with the aim to extend further the knowledge of trans-differentiation of pineal gland into median third eye in trans-plantation set up.

Materials and Methods

The present study was done in two parts. For the first part 80 tadpoles of the toad, *B. melanostictus* of two different stages viz., 40 of external gill stage and 40 five-toe stage were employed. The tadpoles of external gill stage were treated as "donors" and were anaesthetized in a 1: 4000 solution of MS 222 (ethyl-m-aminobenzoate methane sulphonate, Sandoz) before their operation. A fine oblique cut was made to remove the anterior part of cephalic region including both developing eyes, keeping the oral armature intact. For the transplantation purpose, the pineal gland along with a small part of brain was cut out from the operated donor tadpoles by a fine blade under the stereoscopic binocular. Care was taken to leave the major part of the brain intact. The isolated tissue was then grafted into the pit made between the two lateral eyes of the anesthetized five toe stage recipient tadpoles. For making the pit a small incision was made in between two lateral eyes. The incision was made deep up to the brain. Then the tissue (pineal gland along with a small part of brain) was gently inserted into the pit. During the operation the animals were kept on a pad of filter paper moistened with MS 222 solution. After transplantation, each animal was kept immovable for 30-40 min by keeping it partly immersed in diluted MS 222 solution in a petri dish. This allowed time for the graft to be taken. After the implantation, tadpoles were divided into two groups. Half (20) of them were reared in15 IU/ml vitamin-A solution and the remaining 20 were reared in tap water (controls). For the second part of experiment the technique followed was the same but the stage of donor tadpoles was different, i.e., 40 mature 5 toe stage tadpoles were employed (as donors), whereas the stage of recipient tadpoles was kept same (5 toe stage). The experiment was carried out at room temperature (35°-37°C). The method of operation and implantation is shown in Figs 1-3. Following the operation, the tadpoles were preserved in Bouin's solution at different intervals (day 3, 5, 7 and 15) for morphological and histological study. The experiment was terminated on day 15 after operation.

Results

The results are presented in Table 1. These results support previous findings[17-19]. In previous studies median "third eye" was successfully developed after

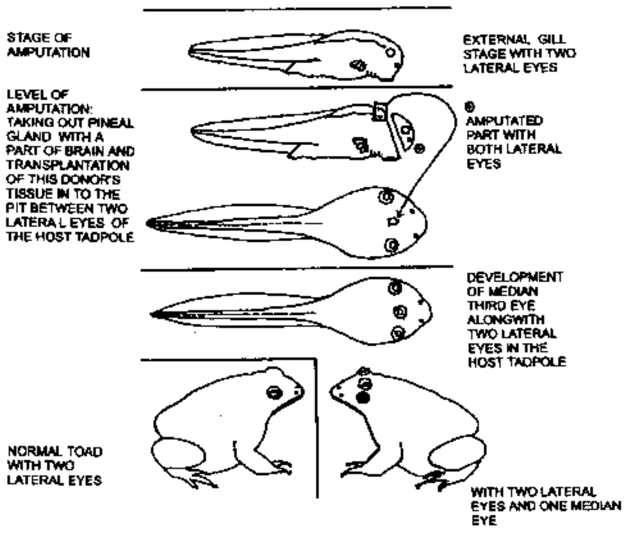
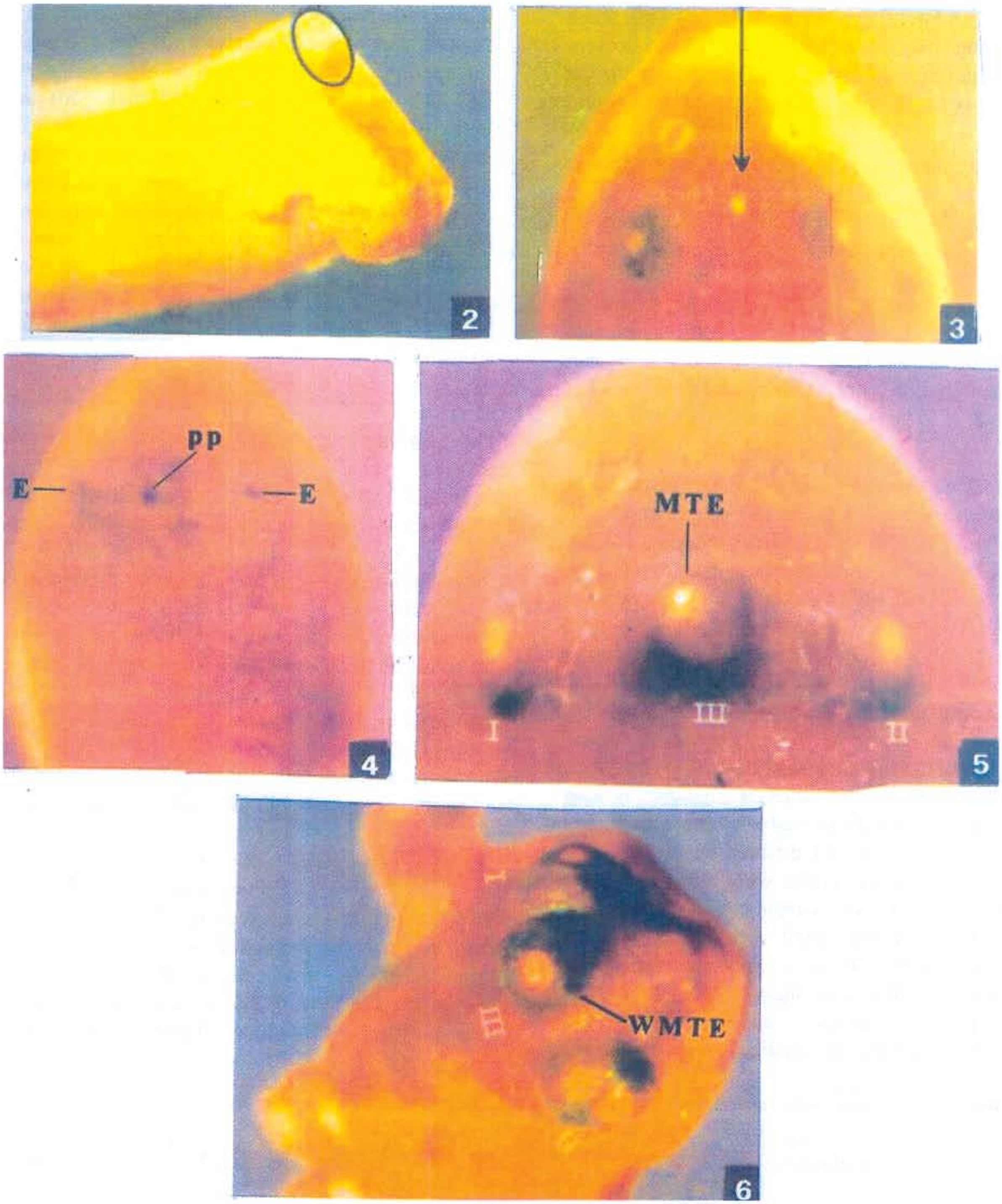


Fig.1 — Line sketch diagram showing the technique of transplantation of donor's pineal gland into the pit made between two lateral eyes of recipient mature tadpole.



Figs 2 and 3-4 — Photograph of external gill stage tadpole of the toad, *Bufo melanostictus* in which anterior cephalic region including two lateral eyes were removed. The circle (Fig.1) shows the position of pineal gland of the donor tadpole and arrow (→) in Fig.2 indicates the position where donor's pineal gland transplanted into the pit made between two lateral eyes of recipient mature tadpole. Fig. 4 — Photograph of a recipient mature tadpole showing development of median third eye from the transplanted pineal tissue (TPT) on day 3 in vitamin A treated group. Fig. 5 — Photograph of a recipient vitamin A treated mature tadpole that received pineal gland implant showing well-developed median third eye (III) in between two original lateral eyes on day 7. Fig. 6 — Photograph of a recipient vitamin A treated metamorphosed toadlet (young toad) showing well-developed median third eye (III). (PP = pigmented protuberance, E = eye, MTE = median third eye, WMTE = well developed median third eye)[Fig. 2: 10 ×; Fig. 3: 5×; Fig. 4: 5×; Fig. 5: 5×; Fig. 6: 5×].

removal of both lateral eyes in early external gill stage tadpoles of the toad, *B. melanostictus*. In the present study, the fate of implants in recipient tadpoles at different intervals of time was observed and found that:

1. A protuberance like structure starts to appear at the site of implantation in controls as well as vitamin A treated tadpoles (Fig.4). Later on by day 5 to 7 after implantation these protuberances trans-differentiated into the median third eye (Fig.5). By day 15 post operation complete median third eye was found to develop in tadpoles of both the groups (Figs 5 and 6). Such a median eye developed in 9 out of 20 cases in controls but in 18 out of 20 cases of vitamin A treated group (Table 1).

2. Although the percentage of median third eye was found high (90%) in vitamin A treated tadpoles in comparison to untreated control tadpoles (45%), structurally the median eyes so developed were found similar in all respects to normal eyes in both. Similar to normal eyes the newly developed median third eyes contain normal cornea, retina and lens.

   Histological findings revealed that the median third eyes so developed were similar to normal intact eyes having normal size with spacious vitreous chamber. The aqueous chamber is also present and bounded externally by the cornea. Iris and retina were found of differentiated state. The visual cells, rods and cones were associated with ganglion layer. In most cases, newly transformed median eye was connected with the brain by a stalk like structure (Fig.7) while in some other cases median third eye show direct connection with the brain (Figs.8 and 9). Thus the histological features of median eye were found almost identical to normal functional eyes. In the second part of the experiment the same technique of operation was performed on older tadpoles (5 toe stage tadpoles). Older tadpoles (40) were employed as donors from which pineal gland along with a small part of brain tissue was taken out and transplanted into the pit made between two lateral eyes of 40 older recipient tadpoles. In these experiments median third eye was not reported even in a single operated tadpole of either controls or vitamin A treated groups; only an undifferentiated and unidentified cellular mass was seen.

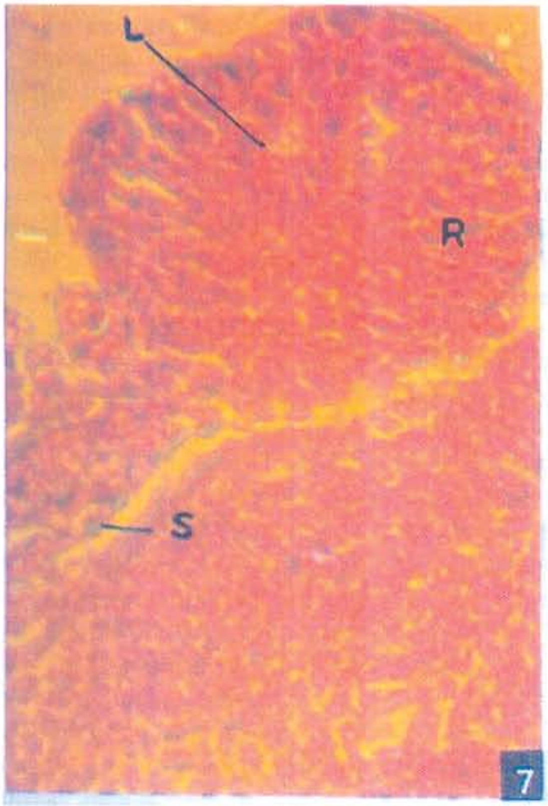
Discussion

The findings are at preliminary stage but interesting. The causal chain behind trans-differentiation of pineal gland into a median third eye is not yet clear. Development of median third eye was found only in early external gills stage tadpoles in previous findings[17,18], but has not been reported in the late stage tadpoles. However, the results of present transplantation technique clearly show that the pineal gland could be transformed into median third eye, and vitamin A enhanced the percentage of this transformation. It seems true as normal lateral eyes and pineal gland both have almost similar embryonic development. Similar to paired eyes the pineal sacs also developed by evagination of the diencephalon roof.

Trans-differentiation (conversion of one cell type to another) generally occurs between cells, which arise from neighbouring region of the same germ layer. *In vitro* the best-studied model for trans-differentiation is the conversion of pigmented epithelial cells of the retina to lens cells so called Wolffian lens regeneration. Eguchi *et al.*[20] also -

Table 1— Development of median third eye from the implanted pineal cells in the tadpoles of toad, *B. melanostictus*

Group	No. of implanted cases	Number of		Number of		Percentage of median third eyes
		Days of preservation after operation	Preserved animals	Median protuberance like structure	Median third eye	
Control group	20	3	3	0	-	
(operated animals		5	3	3	-	45
reared in water)		7	3	0	2	
		15	11	0	4	
Vitamin A treated	20	3	3	2	-	
group (operated		5	3	3	-	
animals reared in		7	3	-	3	90
vitamin A solution)		15	11	-	10	



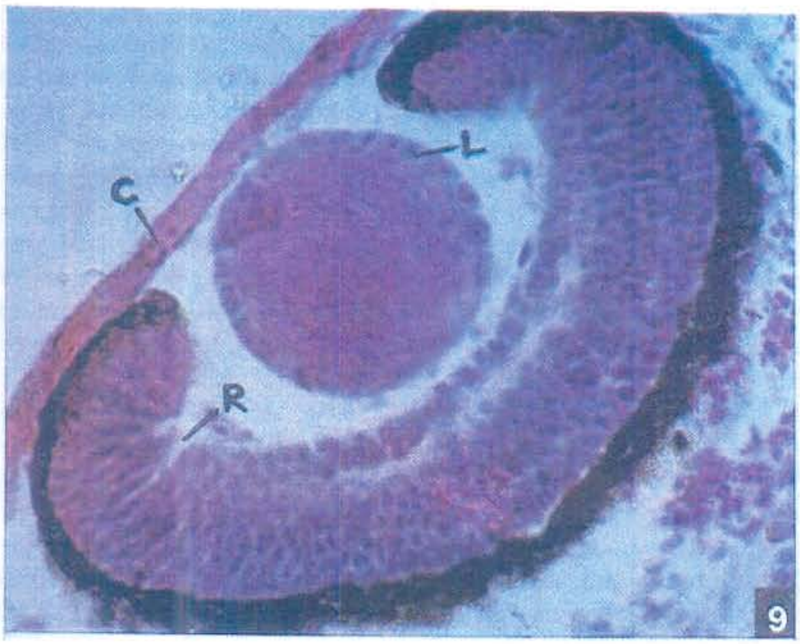


Fig.7 — Microphotograph of a section through the developing median third eye of vitamin A treated tadpole showing early morphogenetic developmental stage of lens (L) and retina (R). A cellular stalk like structure (S) develops between developing median eye and the brain on day 5 after implantation (100 ×). Fig.8 — Microphotograph of a section through median third eye of vitamin A treated tadpole showing further advance differentiation of eye components like lens (L), retina (R) and cornea (C) on day 7 after implantation (100 ×). Fig.9 — Microphotograph of a section through developed median eye of vitamin A treated tadpole showing well differentiated lens (L), retina (R) and cornea (C). (PP = pigmented protuberance, BT = brain tissue, E = eye, MTE = median third eye, WMTE = well developed median third eye)[Fig. 9: 100 ×]

observed that pigmented epithelial cells of avian embryos and even in human fetuses they can readily trans-differentiate into lens cells, when dissociated and cultured *in vitro*. Several environmental conditions controlling the processes have been suggested, particularly the stage of embryonic development. De Pomerai and Clayton[21] also studied the influence of embryonic stage on the trans differentiation of chick neural retina cells in culture. They found that neural retina cells from chick embryos upto 15 days of incubation can trans-differentiate in culture into both lentoids and pigmented cells while no trans-differentiation occurred in cultures of neural retina from embryo immediately before hatching.

The above findings support the present result. In the present study too, the pineal tissue of early external gill stage tadpoles could trans-differentiate into complete median eyes, whereas implants from older tadpoles could not form the median eye. The ciliated cells and supporting cells of the pineal gland become specialized as photoreceptor[22]. They show the same general plan as the paired eyes but with no differentiated lens of the dioptric apparatus. Further, pineal retina of *Tachysurus sona* and also the retina of the lateral eyes may be derived from the photoreceptor rich diencephalon wall of the brain[22]. This accounts for the presence of corresponding auxiliary structures for their photosensory functioning namely a transparent cornea equivalent and a biconvex lens equivalent, be it a lateral eye or a median pineal eye. Srivastava and Srivastava[22] suggested that the pineal retina is very close to the lens in *Tachysurus sona* and therefore, some form of an indirect visual role if not subject imaging is not ruled out such as shadow-imaging of objects moving above the head of the fish in environment exposed to sunshine during day (photo phase, twilight). Thus, the third eye of this living catfish may throw light on its possible primitive function in early vertebrates.

Sivak[23] observed that anatomically well-developed median eye of lamprey (*Petromyzon*) and tuatara (*Sphenodon*) consisted of a lens, a vitreous body and a pigmented retina. The ontogenetic development of the median eye appears to be consistent among the species possessing such an organ. In every case, the median eye is formed as dorsal vesicular outgrowth of the diencephalon, the distal portion of which develops in a lens, while the proximal end develops into a retina. Even in mammals Araki[24] reported that under culture conditions, cells from newborn rat pineals express properties characteristic of photoreceptors. In the pineals of all studied animals, the antibody against the red sensitive cone opsin exclusively recognized the degenerate photoreceptor outer segments[25]. It was suggested that the presence of immunopositive visual pigments indicates the possibility of a retained light sensitivity in the blind cave salamander photoreceptors. At molecular level even the pineal glands were found to be similar to paired lateral eyes in some respects. Casarosa *et al.*[26] studied Xrx-1, a novel *Xenopus* homeobox gene expressed during eye and pineal gland development. They found Xrx-1 is expressed in the anterior neural plate and subsequently in the neural structure of the developing eye (neural retina and pigmented epithelium) and in the pineal gland. Vigh *et al*[27] compared the distribution of immunoreactive glutamate in the pineal complex and retina of frogs and found that there was no essential difference in the immunoreactivity of retina and pineal elements of the species studied. Moreover, Ho *et al.*[16] observed that rodopsin kinase, once thought to be a retinal enzyme, was found at high level in the pineal gland. Melatonin-binding sites have also been demonstrated in the cells of pineal gland as well as in retinal cells of the eye. It has also been observed that pinealocytes of the pineal share common components of signal transduction[28]. More recently, Bellingham *et al.*[29] suggested that photoreceptor is best understood in the retinal rods and cones and in pineal gland as well. Thus, the similarities of pineal gland to paired eyes and previous findings[17,18] give an idea that the pineal gland may be activated and get trans-differentiated into a median third eye. It may be possible that mesenchymal cells of this cephalic region, particularly the eye field region, may induce the epidermal cells to transform into a complete median eye. For trans-differentiation the basic principle is the cell interaction model. Cells are communicating through signals and receptors during the developmental process. One cell signals to reserve cells to make a certain sort of cell which then, when it is made, itself signals to other reserve cells to make yet another type of cell. In this way, specialized cells are developed in a certain order. Signaling genes control the process of cell formation. The signaling factors and receptors are currently being identified. One class of factors is called transforming growth factor beta (TGF beta): a class, which is particularly good at making cells specialize in different directions. Only a very tiny amount (a million millionth of a molar solution) of this factor is needed to switch a cell from one fate to another. In the present study, vitamin A may have activated the TGF beta of pineal organ to form a new median third eye.

As the development of median eye is reported in the tadpoles of both the control and vitamin A treated groups, it is quite possible that the simple cut employed might have induced the cells present surrounding the pineal body to trans-differentiate into the complete median eye. It may also be possible that after the removal of both lateral eyes of this early stage tadpoles, some of the cells concerned with the development of eye remained in the "regeneration territory" or "regeneration field" and may have regenerated a complete eye as reported by Jangir *et al.*[17,18]. As these cells were transplanted along with pineal gland in recipient tadpoles might have trans-differentiated into complete median eye. After removal of both lateral eyes, it is not inevitable that the regeneration be completely repressed if the whole of the regeneration territory is destroyed. Regeneration may occur, but in a different way. In the shrimp *Palinurus*, the eye may regenerate after being removed. The cut, however, must be made through the eye stalk just proximal to the eye and distal to the nerve ganglion lying at the base of the eye. If the cut is made at the base of stalk, so that the ganglion is removed with the eye, the eye will not regenerate; the regeneration blastema develops into an antenna like organ. Thus, the most plausible explanation of such heteromorphosis is that the ganglion and the eye together constitute one regeneration territory. If a part of the territory remains intact, the complete system may be restored. Similarly, for the present observation it may be possible that the pineal body and its surrounding tissues would have been potent to trans-differentiate into complete median third eye.

After discussing the possible similarities between the pineal gland and paired lateral eyes it is appropriate to discuss the role played by vitamin A which increased the percentage of development of median third eye in the present study. Vitamin A and its derivatives, the retinoids, have remarkable effects on various systems in the developing embryo. Synthesis of melatonin in pineal gland is under the control of light environment. Fu *et al.*[30] observed retinal rhodospin in the avian pinealocytes and suggested that vitamin A is involved in photo responses of both the pineal gland and lateral eyes. The most remarkable effects of retinoids, namely the homeotic transformation of tail cells into limbs were reported by Mohanty-Hejmadi *et al.*[31] and later Maden[32,33] confirmed the results and suggested that retinoic acid acts on cells to establish or change the pattern of gene activity. Tini *et al.*[34]also reported that retinoic acid (RA) effect on the eye as a natural endogenous morphogenetic agent and is a regulator of gene expression in the lens. In the present study, too, vitamin A was found to induce pineal gland to transform into median third eye *in situ* (in previous study Jangir *et al*[17,18]) as well as in transplantation condition.

Besides, like other teratogens, vitamin A was also found to show stage dependent effect on developing organs. The origin as well as disappearance of sensitivity to the influence of retinoids are supposed to be the outcome of certain degree of differentiation of tissues[35]. It gives support to the present results of second part of the experiment in which vitamin A could not induce trans-differentiation of pineal implants into median third eye in mature 5 toe stage tadpoles.

Regarding the mechanism, how vitamin A affects the cells to increase the developmental potencies, Maden[36,37] suggested retinoids enter the cells either via unidentified surface receptor or by lipophilic intercalation through the membrane and then binds to cytoplasm binding protein[38]. The complex is then transported to the nucleus where it ultimately alters the pattern of gene transcription perhaps by interaction with receptor proteins. Thus, it can be suggested that retinoids play an important role in the transmission of their ligands from the cell membrane to the nucleus where the pattern of gene activity may be altered. From the present results it can be concluded that similar to the previous findings[17,18]. Vitamin A may have induced and accelerated the implanted pineal gland to transform into the median third eye.

Acknowledgement

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References

1 Hadorn E, Transdetermination, in *The genetics and biology of Drosophila*, edited by Ashburner T & R F Wright, (Academic Press, New York), 1978, 555.

2 Shearn A, Davis K T & Hersperger E, Transdetermination of Drosophila imaginal discs cultured *in vitro*, *Dev Biol*, 65 (1978) 536.

3 Gehring W J & Nothiger R, The imaginal discs of Drosophila, in *Developmental system*, Insect, edited by S J Conch and C H Weddington, (Academic Press, New York), 1973, 211.

4 Kauffman S A, Pattern formation in the Drosophila embryo, *Phil Trans R Soc London Ser B*, 295 (1981) 567.

5 Ouweneel W J, Development genetics of homoeosis, in *Advances in genetics*, edited by M Demerec (Academic Press, New York) 1976, 18.

6 Eguchi G & Kodama R, Transdifferentiation, Review, *Curr Opin Cell Biol*, 5 (1993) 1023.

7 Lo D C, Allen F & Brokes J P, Reversal of muscle differentiation during urodele limb regeneration, *Proc Natl Acad Sci*, 90 (1993) 7230.

8 Mitasov V I, Cell sources, regulatory factors and gene expression during lens and retina regeneration in vertebrates, *Izv Akad Nauk Ser Biol*, 3 (1996) 298.

9 Orii H, Hyuga M, Mochii M, Kosaka J, Eguchi G & Watanabe K, Predominant melanogenesis and lentoidogenesis *in vitro* from multipotent pineal cells by Di methyl sulfoxide and hexamethylene bisacetamide, *Int J Dev Biol*, 38 (1994) 397.

10 Smith H M, *Evolution of chordate structure*, (Holt, Rinehart and Winston Inc. London) 1960, 413.

11 Kelly D E, Ultrastructure and development of amphibian pineal organ, *Progr Brain Res*, 10 (1965) 270.

12 Kelly D E, Developmental aspects of amphibian pineal system, in *The pineal gland, A Ciba Foundation Symposium* (edited by GEW Wolstenholme & J Knight) (Churchill Livingstone,Edinburgh, London) 1971, 101.

13 Hendrickson A E & Kelly D E, Development of the amphibian pineal organ- fine structure during maturation, *Anat Rec*, 170 (1970) 129.

14 Gundy G C & Wurst G Z, Parietal eye- pineal morphology in lizards and its physiological implications, *Anat Rec*, 185 (1976) 419.

15 Sato T, Sensory and endocrine characteristics of the avian organ, *Microsc Res Tech*, 53 (2001) 2.

16 Ho A K, Somers R L & Kelin D C, Development and regulation of rhodopsin kinase in rat pineal and retina, *J Neurochem*, 46 (1986) 1176.

17 Jangir O P, Shekhawat D V S, Acharya P & Suthar P, Compensatory homeotic regeneration of eye in amphibian tadpoles, *Pranikee-J Zool Soc of Orissa*, (Special Volume) (2001) 117.

18 Jangir O P, Shekhawat D V S, Acharya P, Swami K K & Suthar P, Homeotic regeneration of eye in amphibian tadpoles and its enhancement by vitamin A, *J Biosci*, 26 (2001) 577.

19 Suthar P & Jangir O P, *Development of a median "Third eye" in the tadpoles of Bufo melanostictus*, National symposium on development and regeneration, Karnatak University, Dharwad, March 7-8, 2003.

20 Eguchi G, Masuda A, Karasaway, Kodama R & Itoh Y, Microenvironment controlling the trans-differentiation of vertebrate pigmented epithelial cells in *in vitro* culture, *Adv Exp Med Biol*, 158 (1982) 209.

21 De Pomerai D I & Clayton R N, Influence of embryonic stage on the transdifferentiation of chick neural retina cells in culture, *J Embryol Exp Morph*, 47 (1978) 179.

22 Srivastava S & Srivastava C B L, Occurrence of third eye (Pineal Eye) in a living fish (*Tachysurus sona*), *Proc Natl Acad Sci*, India, 68 (1998) 241.

23 Sivak J G, Historical note: The vertebrate median eye, *Vision Res*, 14 (1974) 137.

24 Araki M, Developmental potency of cultured pineal gland cells. An approach to pineal developmental biology, *Microsc Res Tech*, 153 (2001) 33.

25 Kos M, Bulog B, Szel A & Rohlich P, Immunocytochemical demonstration of visual pigments in degenerate retinal and pineal photoreceptor of the blind cave salamander (*Proteus anguinus*), *Cell tissue Res*, 303 (2001)15.

26 Casarosa S, Andreazzoli M, Simeone A & Barsecchi G, Xrx-1, A novel *Xenopus* homeobox gene expressed during eye and pineal gland development, *Mech Dev*, 6 (1997)187.

27 Vigh B, Vigh-Teichmann I, Debreceni K & Takacs J, Similar fine structure localization of immuno-reactive glutamate in the frog pineal complex and retina, *Arch Histol Cytol*, 58 (1995) 37.

28 Okada T S, *Transdifferentiation, flexibility in cell differentiation*, (Clarendon Press, Oxford), 1991, 99.

29 Bellingham J, Whitemore D, Philp A R, Wells D J & Foster R G, Zebra fish smelanopsin, Isolation, tissue localization and phylogenetic position, *Mol Brain Res*, 107 (2002) 128.

30 Fu Z, Kato H, Sugahara K & Kubo T, Vitamin A deficiency reduces the responsiveness of pineal gland to light in Japanese quail (*Coturnix Japonica*), *Comp Biochem Physiol A Mol Intergr Physio*, 119 (1998) 593.

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

32 Maden M, The homeotic transformation of tail into limbs in *Rana temporaria* by retinoids, *Dev Biol*, 159 (1993) 379.

33 Maden M, The role of retinoic acid in embryonic and post-embryonic development, *Proc Nat Soc*, 59 (2000) 65.

34 Tini M, Otulakowski G M L, Tsui L C & Giguere V, An everted repeat mediate retionic acid induction of the gamma F crystalline gene: evidence of direct role for retinoids in lens development, *Genes Dev*, 7 (1993) 295.

35 Niazi I A, Background to work on retinoids and amphibians limb regeneration: Studies on anuran tadpoles – a retrospect, *J Biosci*, 21 (1996) 273.

36 Maden M, *Vitamin A and its effect on pattern formation in the regenerating limb*, Proc 6 M Singer Symposium edited by Inouc *et al*, (1988) 111.

37 Maden M, Retinoids as endogenous components of the regenerating limb and tail, *Wound Repair Regen*, 6 (1998) 358.

38 Chytill F & Ong D E, Cellular retinoid binding protein, in *The retinoids*, edited by M B Sporn and D S Godman, (Academic Press New York) 1984, 89.