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Research Article

HOMEOTIC TRANSFORMATION OF INJURED TAIL TISSUE INTO LIMB SEGMENTS IN TADPOLES OF THE TOAD, *BUFO MELANOSTICTUS* BY VITAMIN A

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ABSTRACT
In this study the effect of vitamin A on injured tail tissue of toad tadpoles was investigated. After making a pit on mid lateral position of tail, tadpoles were exposed to vitamin A solution (15IU/ml) for first 3days and then transferred to water. The results obtained show that vitamin A modify the fate of injured tail tissue to form an additional body segments like pelvic girdle elements and hind limbs. Whereas in untreated control operated animals, simple healing of injured tail was observed. This is an additional report of a homeotic duplication of body segment in vertebrate axial regeneration.

Keywords: *Homeotic, Transformation, Vitamin A*

INTRODUCTION
Vitamin A and its derivative, retinoids are important signaling molecules in development and potent teratogen known to also have a modifying influence on the regeneration of amphibian tails and limbs (Morris-Kay, 1992; Niazi and Saxena, 1979; Jangir and Niazi, 1978; Jangir, 1979; Maden, 1983). Duplication in the antero-posterior (AP) axis of the limb was reported in developing chick treated with retinoic acid (Summerbell, 1983; Tickle *et al.*, 1982). As reported by Niazi *et al.*, (1985) and Maden (1983) retinoids can lead to pattern duplication in proximodistal (PD), AP and DV axes in regenerating amphibian limbs. However, inhibiting effect of retinoids on tail regeneration was reported in *Bufo andersonii* (Niazi and Saxena, 1978). One of the outstanding effects of vitamin A is the ability to induce development of ectopic limbs at the site of tail amputation in anuran tadpoles (Mohanty- Hejmadi *et al.*, 1992; Mahapatra and Mohanty Hejmadi, 1994; Mohanty- Hejmadi and Crawford, 2003; Muller *et al.*, 1996). Since vitamin A inhibits tail regeneration but induces duplication in regenerating limb. Scadding (1987) has opinion that the morphogenetic processes involved in tail regeneration are at least in some way different from those occurring in limbs. In addition to the inhibition of tail regeneration, limbs were generated at the site of amputation in several anuran amphibians (Mohanty-Hejmadi *et al.*, 1992). However, in this paper we report the transdifferentiation/ reprogramming of injured tail tissue (without complete amputation) into legs after vitamin A treatment in toad tadpoles.

MATERIALS AND METHODS
Bufo melanostictus tadpoles were raised from fertilized eggs. Early 5 toe stage tadpoles were employed for experiment. Tadpoles were fed on half boiled spinach leaves. Tadpoles were anaesthetized in 1:2000 MS 222 solution for 3- 5 minutes before operation and fixation. Experiments were conducted at room temperature (35^o- 37^o C). A semicircular pit (1mm deep and 1 mm wide) was made by a sharp sterile needle at mid lateral position (towards the trunk side) of the tail. Complete part of the tail was not amputated rather a small piece of tissue from the pit was extracted (Figure 1). Half of the operated tadpoles were reared in 15IU/ml vitamin A solution for first 3 days and then transferred in tap water. Remaining half of the tadpoles reared in tap water (as control). The experiments were terminated on day 20 after operation. Operated tadpoles of both vitamin A treated and untreated control group were fixed in Bouin’s solution on day 5, 7, 15 and 20 for histological evaluation. Experimental and control regenerates serially sectioned and stained with haemotoxylin and counter stained with eosin.

RESULTS

The results obtained are presented in Table 1. In almost all untreated group tadpoles there was normal healing of injured tail part except 2 out of 30 where a small secondary tail like structure developed (Table 1). There was no structural abnormality observed in control (untreated) group tadpoles. In contrast to untreated controls, the formation of distinct regeneration blastema observed in vitamin A treated tails. The regenerate eventually growing from the blastema developed on injured site (mid lateral position of the tail Figures 2, 3 and 4). Internally, the stump consists mainly of a large notochord, a small neural tube and the musculature (arranged in segmental packages). Ectopic hind limb formation occurred only in vitamin A treated tadpole's tail at injured site. In three, single limb and in 6, paired limbs articulated with pelvic girdle reported out of 14 regenerates. These ectopically developed limbs were small in size with normal morphology consisting thigh, shank foot, ankle and digits. The ectopic hind limbs mostly arose in pairs, only in three cases single limb reported at injured site of the tail. The pattern of skeletal structure of limbs was comparable with those in normal hind limbs. However, the numbers of skeletal elements were reduced in distal parts (digits) in ectopic hind limbs. Formation of pelvic girdle was observed in 9 out of the 30 (Figures 5, 6 and 7). The most striking result of the present study is reporting of presence of skeletal elements in vitamin A treated regenerates whereas anuran tails do not contain a skeleton.

Histological studies revealed a remarkable difference between untreated control and vitamin A treated tadpoles. In untreated tadpoles, the injured pit get healed up completely whereas in vitamin A treated cases, regenerated structure from injured pit on the mid lateral position of the tail lost its tail characteristics and become limb bud like structure (Figures 4 and 5). A thick apical ectodermal cap (Figure 3) was formed in vitamin A treated tadpole after 3 days post operation. Later on it becomes limb bud like structure (Figure 4) and then developed and differentiate into ectopic hind limb by day 20 post operation (Figures 5, 6 and 7). The whole pelvic segments as shown in figures 5, 6, and 7 with skeletal elements which normally do not occur in the tail are generated.

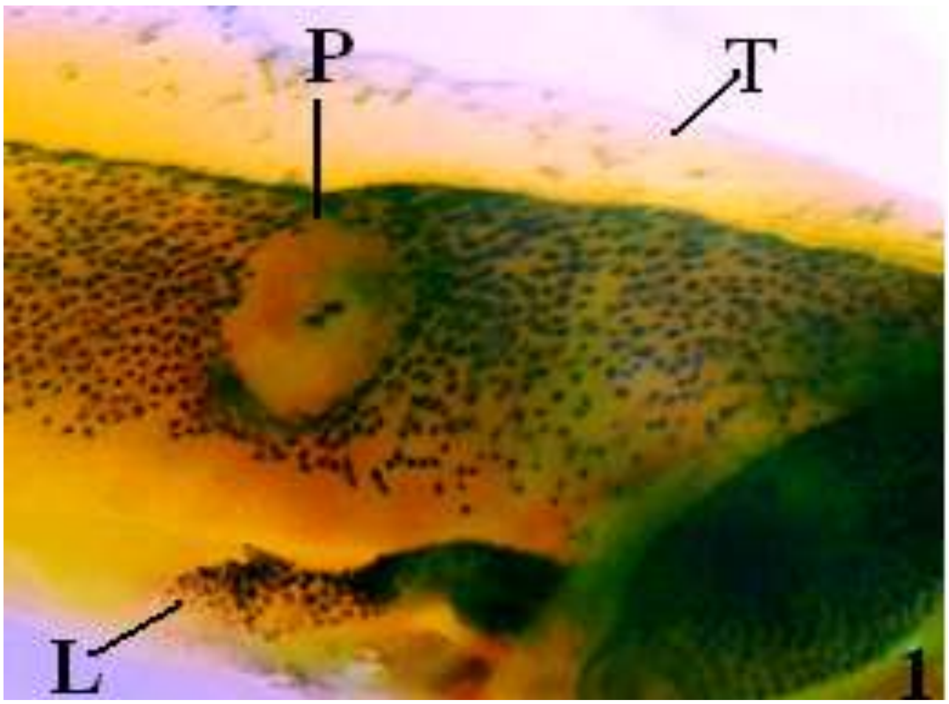

Figure1: Photograph showing the position (mid lateral region) of a pit made on tail of the young tadpole of the toad of *Bufo melanostictus.*

Table 1: Effect of vitamin A (15 IU/ml) on injury- induced plasticity and transdifferentiation of tail tissue into limbs in tadpoles of the toad, *Bufo melanostictus*

Developme-ntal stage of tadpoles employed	Mode of operation	Group (no. of animals employed)	Day of preservation	No. of animals preserved	Number of structure developed						% of ectopic limb development
					Normal healing	Second-ary tail	Ectopic limb bud	Paired Ectopic limb	Single Ectopic limb	Unidentified structure	
Early 5 toe stage Tadpoles	A pit (1mm deep and 1mm wide) made on mid lateral position of tail	Control A (30)	5	5	5	-	-	-	-	-	00%
			7	5	5	-	-	-	-	-	
			15	5	5	-	-	-	-	-	
			20	15	13	2	-	-	-	-	
		Vit. A treated B (30)	5	5	1	-	3	-	-	1	46.66%
			7	5	2	-	1	1	-	1	
			15	5	1	-	-	3	1	-	
			20	15	4	-	1	2	2	6	

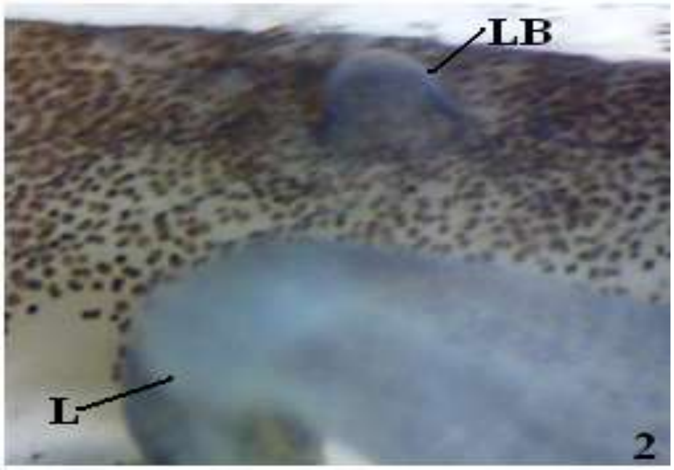

Figure 2: Photograph showing development of limb blastema at the pit made on mid lateral position of a 3 day vitamin A treated tadpole.

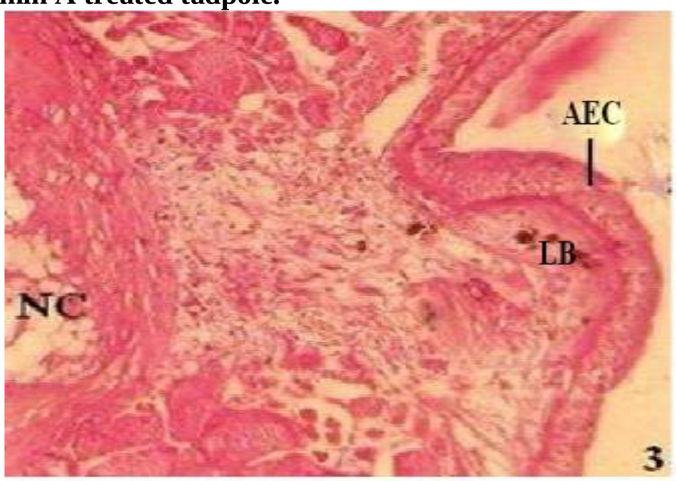

Figure 3: Microphotograph of a section passing through 3 day vitamin A treated tadpole's tail showing development of a limb blastema at the operated site. (40X) Note blastemal cells accumulate beneath the apical ectodermal cap like structure.

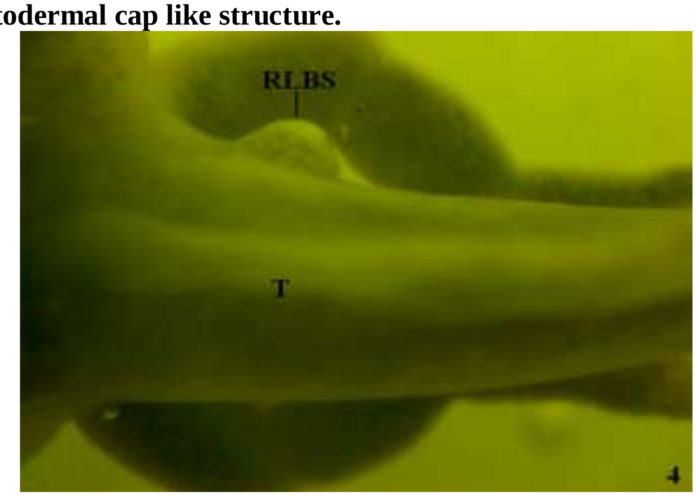

Figure 4: Photograph showing regenerated limb bud like structure developed at the operated site. (Mid lateral position) of 3 day vitamin A treated tadpole's tail

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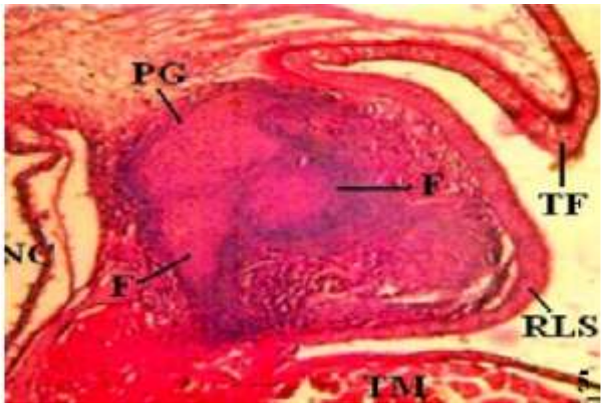

Figures 5: Microphotograph of a section passing through the operated tail of 7 day vitamin A treated tadpole. Note newly developed limb segment at mid lateral position of the tail bearing condensed limb skeletal segments. (40X)

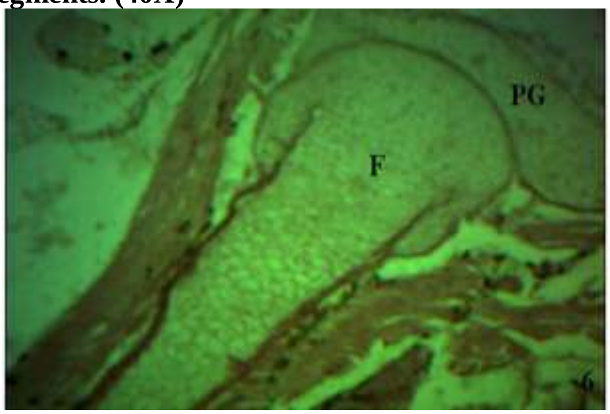

Figure 6: Microphotograph of a section passing through the tail of 20 day vitamin A treated tadpole. Note well defined and differentiated limb structure articulated with pelvic girdle, developed at injury site of the tail. (100X)

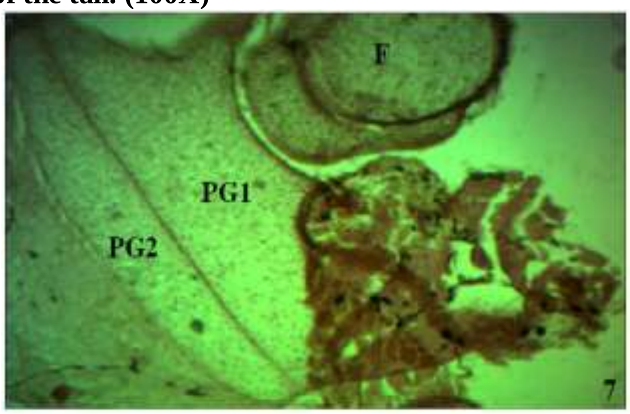

Figure 7: Microphotograph of a section passing through the tail of 20 day vitamin A treated tadpole. Note two well defined pelvic girdles PG1 and PG2 articulated with limb structure developed at injury site of the tail. (100X)

P = Pit made on mid lateral position of tail; T = Tail; L = Limb; LB = Limb Blastema; AEC = Apical Ectodermal Cap; RLBS = regenerated limb bud like structure; PG = Pelvic Girdle; NDL; F= Femur; RLS = Regenerated Limb Structure; TF = Tail Fin; NC = Notochord; TM = Tail musculature

DISCUSSION

The present results give clear evidence of influence of vitamin A on homeotic transformation of injured tail tissue into pelvic elements and limbs. Mahapatra and Mohanty – Hejmadi (1994), Mohanty – Hejmadi and Crawford (2003) also reported homeotic transformation of tail tissue into limb under the influence of retinoids in several species of anuran amphibians. However, they reported development of ectopic hind limbs at the level of amputation of tail. Vitamin A induced ectopic hind limb development from the structure (blastema) developed at the cut ends of the tail. But in present experiment tail was not amputated completely rather a small pit was made on mid lateral position. This was the site of development of ectopic hind limbs in vitamin A treated tadpoles. The present findings give clear evidence of inductive influence of vitamin A on injury induced reprogramming ability of tail tissue into axial skeletal elements, pelvic segments and limbs.

For such type of homeotic transformation of tail into hind limbs, it is reasonable to speculate that under the influence of vitamin A, mesodermal cells in the tail region induce a limb bud in the presence of a mesenchymal FGF which in turn induces Pitx I which acts as a Tbx4 inducer to form the hind limbs (Pitx and Tbx proteins are transcription factors and their targets include genes of Hox homeodomain cluster). Tbx 4 induces hind limb specific Hox genes and suppresses the fore limb specific Hox d9 genes (Niwander, 1999). Mahapatra and Mohanty- Hejmadi (1994) also suggested that a gene of Hox 3 cluster may be involved in the development of the tail to legs. Johnson and Tabin (1997) discussed the role of retinoids in the regulation of Hox genes with respect to development of limbs from tail. Regarding the transformation of tail tissue into a pelvic segments Christ *et al.*, (2000) presented a comprehensive account and explained the role of Hox genes in development of axial and paraxial skeletal elements. It is also known that retinoids can induce axial transformation during early mammalian development (Kessel and Gruss, 1991; Kessel, 1992; Rutledge *et al.*, 1994). It is shown here that retinoids have the capacity to induce the supernumerary formation of a limb segments including more than one tissue, in amphibian regeneration.

In the present study, most noteworthy is the enlargement of the notochord in the vitamin A treated group. In control group a normal notochord was observed after complete healing of injured site whereas in the treated group there is considerable enlargement of notochord which remains in this condition for long time. 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. In chick and mouse, notochord is known to be a source of polarizing factor that is implicated in interaction that pattern the CNS, somite and limb (Martie *et al.*, 1995). As reviewed by Christ *et al.*, (2000), somite formation is not possible without the nerve cord and the notochord. At the injury site of tail both the nerve cord and notochord are present and in vitamin A treated cases considerable enlargement of notochord reported. Therefore, it can be speculated that under the influence of vitamin A, notochord mediates the expression of key Hox genes for the establishment of the pelvic region and nerve supply by respecification and proximalisation, in the tail tissue. These studies on the role of vitamin A in reprogramming/ transdifferentiation/ homeotic transformation of one tissue into another will continue to make a major contribution to understanding of the fundamental problem in developmental biology.

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