The effect of Diethylstilbesterol (DES) and 17-beta- Estradiol (E2) on the growth and tissue chemistry of Labeo rohita. S.K.Kommu, K.R.Shah, V.Arul, and S.A.Bhatt

Research Paper

THE EFFECT OF DIETHYLSTILBESTROL (DES) AND 17- β – ESTRADIOL (E_2) ON THE GROWTH AND TISSUE CHEMISTRY OF *LABEO ROHITA*

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ABSTRACT

Diethylstilbestrol (DES) and 17-β- estradiol (E_2) have shown an increase in the growth rate, food conversion efficiency, total protein content, Protein/DNA and RNA/DNA ratios in the Indian Major Carp, *Labeo rohita*, cultured under natural photo period and temperature. However, no significant change was observed in the total lipid and carbohydrate concentrations. From the results it may be concluded that DES and E_2 reduce the metabolic rate and enhance the accumulation of proteins in tissues. The optimum dosage for *Labeo rohita* is 3-5 ppm for DES and 1-3 ppm for E_2. However DES has a toxic effect with the increase in concentration of the hormone. Diethylstilbestrol and 17-β-estradiol may be included in the anabolic growth promoter and appetite depressant estrogens.

Key words: Diethylstilbestrol, 17- β- estradiol, *Labeo rohita*, aqua culture.

Introduction

In developing nations majority of the population suffer from either malnutrition or ill nutrition. To redress this problem these should be supplied with nutritious food that is economical and fishes, which are rich in nutrients, are an example of such type. In fish hatcheries, increased operational cost has led to the search for technology to maximize the yield within the shortest possible time. This may be achieved using hormonal growth promoters, since hormones are concerned with the control and regulation of energy metabolism and growth (Matty and Lone, 1979). According to Donaldson *et al.* (1979) by using one or more hormones fish can be grown to a larger size with in a shorter span and food conversion efficiency can be improved.

The basic strategy in intensive aquaculture is to maximize growth at minimum feed cost with an end product having high nutritive value and acceptability to the consumer (Donaldson *et al.*, 1979). To promote growth of fish, Pituitary growth hormones, anabolic steroid hormones and thyroid hormones or their synthetic analogues either individually or in different combinations have been applied. Pickford (1954), Swift (1954), Mc Bride and Fagerlund (1973), Higgs *et al.* (1976), Higgs *et al.*(1977), Higgs and Eales (1979) and Matty and Lone (1979) have evaluated the effect of the administered hormone on the growth of fish, while Cowey *et al.* (1973), Mc Bride and Fagerlud (1976), Mc Bride *et al.* (1978), Yu *et al.* (1979), Pandian (1982), Nirmala and Pandian (1983), Sindhu and Pandian, (1984) and Arul (1986) have studied the effect of the hormone on the feeding and growth of fish. The external administration of steroids and xenobiotics influences the sex differentiation in fishes and hence enhances their live weight (Baroiller *et al.*,1999 ; Jalabert *et al.*, 2000).

Ashby (1957) found that the growth of brown trout was much retarded by estradiol and casualities were heavy. However *Silurus asotus* treated with the same estrogen, 17-β- estradiol, has shown increased growth over normal males (Kim *et al.*, 2001). Ghittino (1970) reported that synthetic estrogen, diethylstibestrol (5 – 500 g.100 kg^{-1} feed) fed to rainbow trout slightly depressed the growth. The same estrogen was also shown to lower the

growth rate of catfish (Bulkley, 1972). However, Cowey *et al.* (1973) reported that Diethylstilbestrol (600 mg.kg^{-1} moist feed) fed to Plaice resulted in the increased weight gain, food consumption and food conversion. Similar results were obtained by *Yu et al.* (1979) when estradiol was administered to Coho Salmon. Nirmala and Pandian (1983) also reported that DES increased growth rate and food conversion efficiency by decreasing the food consumption and metabolic rate.

The results of Powers and Florini (1975) and Scott (1978) have confirmed that both estrogens and androgens exert marked effect on growth processes. The body growth is accomplished by protein accretion and cell proliferation. As the protein accretion is function of protein synthesis and protein degradation, for studies like this estimation of nucleic acids (RNA and DNA) and their ratios (RNA/DNA) are required and also these are an 7 integral part of the study (Enesco and Leblond, 1962).

As there is no sufficient information available on the roles of the Diethylstilbestrol and 17-β- estradiol on the growth of *Labeo rohita*, the present investigation was undertaken

1. To identify the optimum dosage of Diethylstilbestrol and 17-β- estradiol on the Indian major carp - *Labeo rohita*.
2. To know whether the hormones exhibit any anabolic property and
3. To know their effect on tissue chemistry.

Materials and Methods

For this study 9 groups (in each 10 fish) of *Labeo rohita* (3.5 ±1.0 g) were selected. To one group (control) no hormone was administered. To 4 groups 1, 3, 5 and 10 ppm of 17-β-estradiol (E_2) sprayed feed was given. To the remaining 4 groups 1, 3, 5 and 10 ppm of DES sprayed feed was given. The above feed was given twice a day for 45 days. The live weights of the fishes were determined on the 46th day and were frozen till further analysis for various parameters *viz.*, protein, carbohydrates, lipid and nucleic acid contents using the tissue homogenate.

Gross food conversion efficiency was calculated using the formula

$$\text{Conversion Efficiency \%} = \frac{\text{Growth} \times 100}{\text{Food Consumed}}$$

Proteins were estimated using modified Lowrys method. Lipid content was estimated by Raymonds method. Anthrone method of Plummer was followed for estimating Carbohydrates. DNA and RNA were estimated using Diphenylamine and Orcinol methods, respectively. The results obtained were subjected to Standard deviation, Student's 't' test and analysis of variance (ANOVA).

Results

The values of effects of DES and E_2 administrations on food conversion efficacy, protein and lipid concentrations, RNA/DNA ratio, carbohydrate concentration and protein/RNA as well as protein/DNA ratios were depicted in Tables 1, 2, 3, 4 and 5, respectively. Fig. 1 shows the weight gain and food conversion efficiency of DES and E_2 graphically.

Table 1

The effect of DES and E_2 on live weight, food conversion efficiency in *Labeo rohita*. Each value represents the average of ten fish (Mean).

Group	Name of the Hormone	Dosage (mg.kg^{-1} feed)	Gain in live weight (%)	Food conversion Efficiency	Survival (%)
1.	control	0.0	0	14.86	90
2.	DES	1.0	68	28.58	90
3.	DES	3.0	77	29.42	80
4.	DES	5.0	70	28.76	80
5.	DES	10.0	67	23.16	70
6.	E_2	1.0	233	38.38	100
7.	E_2	3.0	216	36.52	90
8.	E_2	5.0	191	32.94	100
9	E_2	10.0	180	31.22	100

(DES- Diethylstilbestrol, E_2- 17-β- estradiol)

Table 2

The effect of DES and E_2 on protein and Lipid concentrations in estrogen treated *Labeo rohita*. Each value represents the average of ten fish (Mean ± SD).

Group	Hormone/ control	Dosage ($mg.kg^{-1}$ feed)	Protein (mg/100mg)		Lipid (mg/100mg)	
			Initial	Final	Initial	Final
1.	Control	0.0	17.371 ± 0.55	20.32 ± 0.67	2.10 ± 0.08	2.23 ± 0.09
2.	DES	1.0	17.371 ± 0.55	25.18 ± 0.39*	2.10 ± 0.08	2.23 ± 0.09
3.	DES	3.0	17.371 ± 0.55	27.82 ± 0.40*	2.10 ± 0.08	2.50 ± 0.08
4.	DES	5.0	17.371 ± 0.55	29.73 ± 0.36*	2.10 ± 0.08	2.67 ± 0.07
5.	DES	10.0	17.371 ± 0.55	27.63 ± 0.42*	2.10 ± 0.08	2.42 ± 0.09
6.	E_2	1.0	17.371 ± 0.55	26.18 ± 0.45*	2.10 ± 0.08	2.33 ± 0.05
7.	E_2	3.0	17.371 ± 0.55	27.85 ± 0.49*	2.10 ± 0.08	2.60 ± 0.08
8.	E_2	5.0	17.371 ± 0.55	31.70 ± 0.62*	2.10 ± 0.08	2.63 ± 0.09
9	E_2	10.0	17.371 ± 0.55	27.09 ± 0.45*	2.10 ± 0.08	2.53 ± 0.12

* P < 0.01 (DES- Diethylstilbestrol, E_2- 17-β- estradiol, SD- Standard deviation)

Table 3

The effect of DES and E_2 on RNA/DNA ratios in *Labeo rohita*. Each value represents the average of ten fish (Mean ± SD).

Group	Hormone/ control	Dosage ($mg.kg^{-1}$ feed)	RNA/DNA	
			Initial	Final
1.	Control	0.0	2.70 ± 0.009	3.24 ± 0.035
2.	DES	1.0	2.70 ± 0.009	3.90 ± 0.017*
3.	DES	3.0	2.70 ± 0.009	4.24 ± 0.022*
4.	DES	5.0	2.70 ± 0.009	4.54 ± 0.022**
5.	DES	10.0	2.70 ± 0.009	4.13 ± 0.005*
6.	E_2	1.0	2.70 ± 0.009	3.36 ± 0.006*
7.	E_2	3.0	2.70 ± 0.009	3.75 ± 0.055***
8.	E_2	5.0	2.70 ± 0.009	4.11 ± 0.005**
9	E_2	10.0	2.70 ± 0.009	3.63 ± 0.006**

* P < 0.05, ** P < 0.01, *** P < 0.09
(DES- Diethylstilbestrol, E_2- 17-β- estradiol, SD- Standard deviation)

Table 4

The effect of DES and E_2 on carbohydrate concentration in *Labeo rohita*. Each value represents the average of ten fish (Mean±SD).

Group	Hormone/ control	Dosage ($mg.kg^{-1}$ feed)	Carbohydrate (mg/100mg)	
			Initial	Final
1.	Control	0.0	0.98 ± 0.008	0.98 ± 0.008
2.	DES	1.0	0.98 ± 0.008	0.98 ± 0.008
3.	DES	3.0	0.98 ± 0.008	0.98 ± 0.008
4.	DES	5.0	0.98 ± 0.008	0.98 ± 0.008
5.	DES	10.0	0.98 ± 0.008	0.98 ± 0.008
6.	E_2	1.0	0.98 ± 0.008	0.98 ± 0.008
7.	E_2	3.0	0.98 ± 0.008	0.98 ± 0.008
8.	E_2	5.0	0.98 ± 0.008	0.98 ± 0.008
9	E_2	10.0	0.98 ± 0.008	0.98 ± 0.008

(DES- Diethylstilbestrol, E_2- 17-β- estradiol, SD- Standard deviation)

Table 5

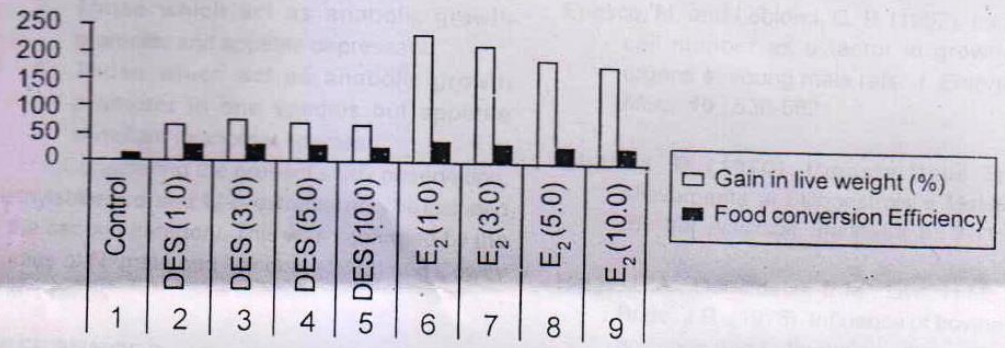
The effect of DES and E_2 on Protein/RNA and Protein/DNA ratios in *Labeo rohita*. Each vaiue represents the average of ten fish (Mean±SD).

Group	Hormone/ control	Dosage ($mg.kg^{-1}$ feed)	Protein/RNA		Protein/DNA	
			Initial	Final	Initial	Final
1.	Control	0.0	69.34 ± 1.91	67.83 ± 1.74	187.42 ± 5.06	219.26 ± 6.04
2.	DES	1.0	69.34 ± 1.91	71.59 ± 0.76	187.42 ± 5.06	279.59 ± 3.78**
3.	DES	3.0	69.34 ± 1.91	69.62 ± 0.71	187.42 ± 5.06	295.22 ± 4.16**
4.	DES	5.0	69.34 ± 1.91	74.47 ± 0.90	187.42 ± 5.06	338.41 ± 5.07**
5.	DES	10.0	69.34 ± 1.91	68.57 ± 0.78	187.42 ± 5.06	283.12 ± 3.38**
6.	E_2	1.0	69.34 ± 1.91	78.59 ± 0.43*	187.42 ± 5.06	265.94 ± 3.02**
7.	E_2	3.0	69.34 ± 1.91	77.58 ± 0.42*	187.42 ± 5.06	290.82 ± 2.83**
8.	E_2	5.0	69.34 ± 1.91	75.90 ± 0.63*	187.42 ± 5.06	311.81 ± 2.71**
9	E_2	10.0	69.34 ± 1.91	79.44 ± 0.52*	187.42 ± 5.06	293.94 ± 3.12**

* P < 0.05, ** P < 0.01 (DES- Diethylstilbestrol, E_2- 17-β- estradiol, SD- Standard deviation)

Fig. 1

Gain in live weight and food conversion efficiency in *Labeo rohita* fed with hormone sprayed feed.



(DES- Diethylstilbestrol, E_2- 17-β- estradiol, SD- Standard deviation)

Discussion

From the results it can be concluded that both the hormones (DES and E_2) promote growth and increase in food conversion efficiency in *Labeo rohita* when compared with the control (Table1). Comparing the effects of DES and E_2 on the food conversion efficiency, it is apparent that E_2 is better than DES (Table 1 and Fig.1). The increase in food conversion efficiency after feeding estrogenic steroids has been reported in other fishes as well (Cowey *et al.*, 1973; Yu *et al.*, 1971 and Nirmala and Pandian, 1983). The exact mechanism behind this enhanced efficiency is yet to be unrevealed. However, studies of Yamazaki (1976) and Nirmala and Pandian (1983) suggest that this effect may be due to induction of digestive enzyme synthesis and / or due to better assimilation.

The feeding of DES and E_2 brought different changes in the tissue chemistry of the fish. These changes are characterized by an increase in tissue total proteins (Table 2), RNA/DNA ratio (Table 3) and Proteins/DNA ratios (Table 5). However no significant change was observed in total carbohydrate content (Table 4) and lipid content (Table 2) in hormone treated fish when compared with the control group. All these parameters are used as growth indicators in different organs (Allen *et al.*, 1979). In fish as growth is reflection of simple accretion of proteins, the changes in the nucleic acids, which are organizers of protein synthesis, are bound to occur. However, it is not clear whether the increase in protein is due to active protein biosynthesis or reduced degradation of proteins in the tissues. Various studies in mammals clearly show that anabolic steroids reduce the degradation of proteins in different tissues of the body (Mayer and Rosen, 1977; Young and Plusleal, 1977; Vernon and Buttery, 1978 and Buttery *et al.*, 1979).

In mammals, the anabolic androgenic steroids increase the nucleic acids, RNA/DNA and Proteins /DNA ratio (Petrovic *et al.*, 1977; Mainwaringh, 1977). It has been reported that normal growth of fish is accomplished by certain biochemical changes and particularly in nucleic acids (Mustafa and Jafri, 1977). According to Kays (1978), Kays (1979) and Medda and Ray (1979) anabolic hormones increase the protein and RNA in liver and muscle of the fish.

Pandian (1982) summarized the available endocrine hormones that influence somatic growth of commercially important fishes into three groups.

1. Those which promotes growth by acting as appetite stimulants.
2. Those which act as anabolic growth promoter and appetite depressant.
3. Those which act as anabolic growth promoter in one species but appetite stimulant in another species.

Considering the present study observation, Diethylstilbestrol and 17-β-estradiol may be included in the second category. This was confirmed by the studies of Nirmala and Pandian (1983) and Cowey *et al.* (1973).

REFERENCES

Allen, R.E., Merkel, R.A. and Young, R.B. (1979). Cellular aspects of muscle growth - Myogenic cell proliferation. *J. Anim. Sci.* **49** : 115-127.

Arul, V. (1986). Effect of ethylesterol on growth and food utilization in *Channa striatus*. *Proc. Indian Acad. Sci.* **95** : 51-58.

Ashby, K.R. (1957). The effect of steroid hormones on the brown trout (*Salmo trutta L.*) during the period of gonadal differentiation. *J. Embryol Exp. Morphol.* **5** : 225-249.

Baroiller, J.F., Guiguen, Y. and Fostier, A. (1999). Endocrine and environmental aspects of sex differentiation in fish. *Cell Mol. Life Sci.* **55** : 910–931.

Bulkley, R.V. (1972). Diethylstilbestrol in catfish feed. *Trans Am. Fish. Soc.* **101**: 537-539.

Buttery, P.J., Vernon, B.G. and Pearson, J.T. (1979). Anabolic agents – some thoughts on their mode of action. *Proc. Nutr. Soc.* **37**: 311-316.

Cowey, C.B., Pope, J.A., Andron, J.W. and Blair, A. (1973). The effect of oral administration of diethylstilbestrol and cyproheptadene on the growth of *Pleuronectes platessa*. *Mar. Biol.* **19**:1-6.

Donaldson, E. M., Fagerlund, U.H.M., Higgs, D.A. and Mc Bride, J. R. (1979). Hormonal enhancement of growth in fish physiology. Vol.8. Newyork: Academic press. pp. 456-498.

Enesco, M. and Leblond, C. P. (1962). Increase in cell number as a factor in growth of the organs of young male rats. *J. Embryol. Exp. Morp.* **10** : 530-562.

Ghittino, P. (1970). Riposte Delle Trote de Allevaments al Stilboestrols e Metiltiuracile. *Riv. Ital. pisicisolt. Ithiopatol.* **5** : 9-11.

Higgs, D.A., Donaldson, E.M., Dye, H.M. and Mc Bride, J.R. (1976). Influence of bovine growth hormone and L- thyroxine on growth, muscle composition and histological structure of the gonads, thyroid, pancreas and pituitary of coho salmon (*Onchorhynchus kisutch*). *J. Fish. Res. Board Can.* **33** : 1585-1603.

Higgs, D.A., Donaldson, E.M., Dye, H.M. and Mc Bride, J.R. (1977). Influence of combination of bovine growth hormone, 17X methyltestosterone and L-thyroxine on growth of yearling coho salmon (*Onchorhynchus kisutch*). *Can. J. Zool.* **55** : 1048-1056.

Higgs, D.A., and Eales, J.G. (1979). Influence of diet composition on radiothyroxine kinetics in brook trout, *Salvelinus fontinalis*. Mitchill *Can. J. Zool.* **57** : 396-402.

Jalabert, B., Baroiller, J. F., Breton, B, Fostier A, Le Gac, F., Guiguen, Y. and Monod, G. (2000). Main neuro-endocrine, endocrine and paracrine regulations of fish reproduction, and vulnerability to xenobiotics. *Ecotoxicology.* **9** : 25–40.

Kays, T. (1978). Effect of hypophysectomy and beef growth hormone replacement therapy on morphometric and biochemical indicators of growth in fed versus starved black bull head (*Ictalurus melas*). *Gen.Comp. Endocrinol.* **35** : 419-431.

Kays, T. (1979). Effect of hyphophysectomy and beef growth hormone replacement therapy over time and at various dosages, on body weight and total RNA-DNA levels in the black bull head (*Ictalurus melas*). *Gen.Comp. Endocrinol.* **37** : 321-332.

Kim, D.S., Cho, H.J., Bang, I.C., Choi, G.C. and Nam, Y.K. (2001). Effects of immersion of larvae in oestradiol-17β on feminization, structural changes of gonad and growth performance in the Far Eastern catfish *Silurus asotus* Linn. *Aquaculture Research.* **32** (5) : 323.

Maiwaringh, W.I.P. (1977). The mechanism of action of androgen. *Mongr. Endocrinol.* **10** : 178.

Matty, A.J. and Lone, K.P. (1979). The effect of androgenic steroids as dietary additives on the growth of carp (*Cyprinus carpio*). *Proc. 10th Annual work shop world Mariculture Soc.* **10** : 735-745.

Mayer, M. and Rossen, F. (1977). Interaction of glucocorticoids and androgens with skeletal muscle. *J. Metabolism.* **26** : 937-962.

Mc Bride, J.R. and Fagerlund, U.H.M. (1973). The use of 17X-methyltestosterone for promoting weight in juvenile Pacific Salmon. *J. Fish. Re. Board Can.* **30** : 1099-1104.

Mc Bride, J.R., and Fagerlud, U.H.M. (1976). Sex steroids as growth promoters in the cultivation of juvenile coho salmon (*Onchorhynchus kistuch*). Proc. 7th Annual workshop World Mariculture Society, January 25-29, San Diego California. Lousian State Univerrsity Press, Batopn Rouge, pp. 145-161.

Mc Bride, J.R., Higgs, D.A. and Fagerrlund, U.H.M. (1978). Influence of feeding a diet containing 17X-methyltestosterone or testosterone at two ration levels on growth, apetite and food conversion efficiency of under yearling coho salmon (*Ophicephalus punctatus*). J.E. Halver and Ktiews, Finish nutrition and fish feed technology, Vol.1 (Berlin: Heenemannverslagagesellschaft). pp. 221-230.

Medda, A.K., and Ray, A.K. (1979). Effect of thyroxine and analogues on protein and nucleic acid contents of liver and muscle of lata fish (*Ophicephalus punctatus*). *Gen. omp. Endocrinol.* **37** : 74-80.

Mustafa, S. (1977). Nucleic acid turnover in dark and white muscles of some fresh water species of carps during growth in the pre-maturity phase. *Copeia.* **1** : 173-176.

Mustafa, S. and Jafari, A.K. (1977). RNA and protein cotents in flesh of the telost *channa punctatus* Bloch during growth. *Annls. Biol. Anim. Biochem. Biophys.* **17** : 991-996.

Nirmala, A.R.C. and Pandian, T.J. (1983). Effect of steroid injection and food utilization in *Channa striatus*. *Proc. Indian Acad. Sci. (Aniur Sci.).* **92** : 221-229.

Pandian, T.J. (1982). Contribution to bioenergetics of tropical fish, Gutshop, (Seattle: Washington University Press), pp. 1-11.

Petrovic, S.L., Novakovice, M.B., Tepvac, R.I., and Wilson, C.W. (1977). Androgen induced accretion of ribonucleic acids in kidney of female mouse (*Mus musculus*). *Int. L. Biochem.* **8** (3) : 193-198.

Pickford, G.E. (1954). The response of hypophysectomized male killfish to purified fish growth hormone, as compared with the response to purified beef growth hormone. *Endocrinology.* **55** : 274-278.

Powers, M.L and Florini, J.R. (1975). A direct effect of testosterone on muscle cells in tissue culture. *Endocrinology.* **97**: 1043-1047.

Raymond, J.R.C., Austin, J. and Linford, E. (1964). Biochemical studies on marine zooplanktons. The biochemical composition of Neomysis inteter. *J. Cons. Int. Explor. Mer.* 354-363.

Scott, B.M. (1978). The use of growth promoting implants in beef production. A.D.A.S. *Quart. Rev.* **31**: 185-216.

Sindhu, S. and Pandian, T. J. (1984). Effect of administering different doses of 17 X methyltestosterone in *Hetropneustes fossilis*. *Proc. Indian Acad. Sci. (Anim. Sci.)*. **93** : 511-516.

Swift, D.R. (1954). Influence of mammalian growth hormone on rate of growth of fish. *Nature (Lon.)*. **173** : 106.

Vernon, B.G. and Buttery, P.J. (1978). Effect of trenbolone acetate with time on various responses of protein synthesis of rat. *Brit. J. Nutr.* **40** : 563-572.

Yamazaki, F. (1976). Application of hormones in Fish Culture. *J. Fish Res. Board Can.* **33** : 948-958.

Yu, T.C., Sinnhuber, R.O. and Hendricks, J.D. (1979). Effect of steroid hormones on the growth of juvenile coho salmon (Onchorhynchus kisutch). *Aquaculture*. **16** : 351-359.

Young, V.R. and Plusleal, M.G. (1977). Mode of action of anabolic agents with special references to steroids and skeletal muscle. A summary review. Proc. 2nd. *Int.Symp. Protein Metab. Nutr.* pp. 17-28.

SMC (STRUCTURAL MAINTENANCE OF CHROMOSOME) PROTEINS: REVIEW ARTICLE

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Abstract:

Structural maintenance of chromosome (SMC) proteins, along with other non SMC elements (Nse) form specific complexes that organize, stabilize, segregate and repair chromosomes. The proper segregation of genetic material during cell division is very vital since defects in the chromosomal DNA segregation may lead to several chromosomal aberrations some of which are lethal. In eukaryotes Cohesin is a ring shaped protein complex, formed by SMC1/3 hetero dimmer. It is required for sister chromatid cohesion and proper positioning of mitotic spindle. Condensin, a complex comprising of SMC2/4 hetero dimer organizes the chromosomes in eukaryotes and helps in their segregation in to daughter cells. Eukaryotic SMC5/6 complex is relatively less studied and is found to have a role in DNA repair though the precise mechanism is not yet understood completely. Prokaryotic SMC proteins have a related though not identical function with their eukaryotic counterparts.

Keywords: *SMC proteins, condensisn, cohesion, Non SMC proteins, Kleisins*

1. INTRODUCTION

The genetic material of all living organisms is DNA. In eukaryotes within the nucleus DNA forms a reticulate network, known as chromatin. This network condenses to form short structures known as chromosomes that are involved in processes such as replication, segregation and repair (Losada & Hirano, 2005). How the reticulate network of chromatin condensed into short chromosomes was an unanswered question for a long time. The DNA replicates during synthesis (S) phase of cell cycle and is equally distributed to the daughter cells during anaphase. However, the replicated DNA in the form of chromosomes remains together until the cell transits from metaphase to anaphase, during which they split and migrate to different poles. What tethers them and what releases them in anaphase was an unanswered question for a long time. However, the discovery of Structural Maintenance of Chromosome proteins has provided some clues to these unanswered questions (Losada & Hirano, 2005; Jessberger, 2002; Uhlmann, 2004).

2. SMC PROTEINS

SMC proteins were first observed in a mutant Escherichia coli with impaired chromosome segregation. These SMC proteins are seen in all living organisms (Cobbe & Heck, 2004). In majority of the prokaryotes and archea a single SMC protein that forms a homo dimer is seen (Hiraga, 2000). However in eukaryotes at least six SMC proteins SMC1-6 are seen. These proteins form specific hetero dimers namely SMC1/3,

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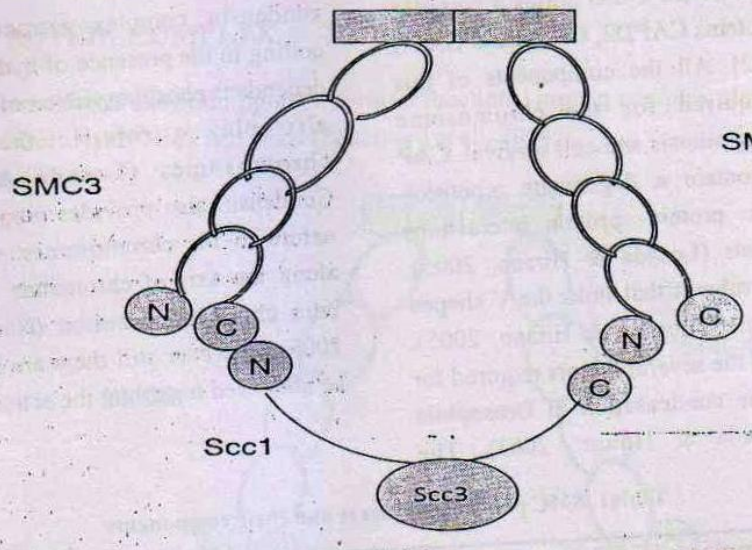

Figure3 showing the structure of cohesin complex in which SMC1 &3 heterodimer is linked by alpha kleisin, Scc1. Scc3 is a Nse linked to Scc1 (N amino terminus, C carboxyl terminus).

6. SMC2/4 PROTEIN COMPLEX- CONDENSIN

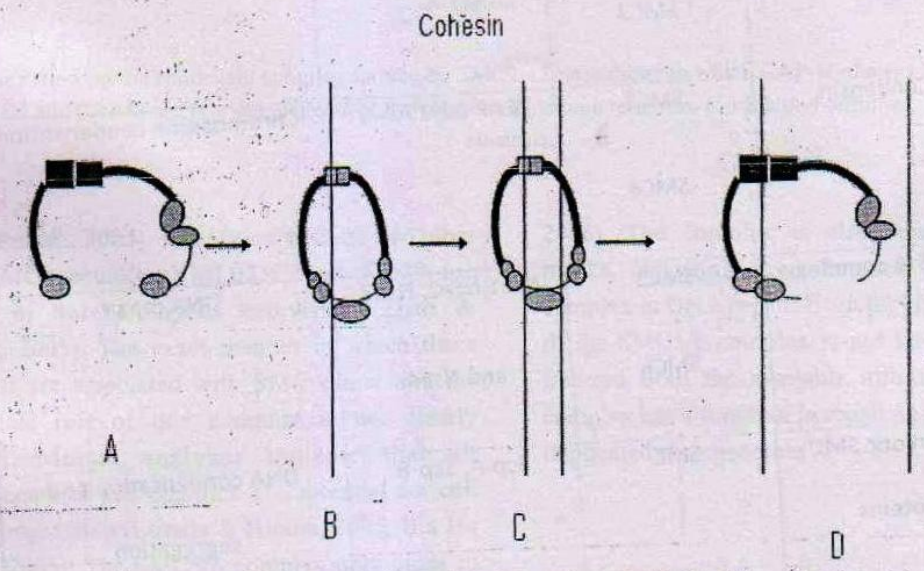

Figure4 showing the ring model proposed to explain the action of cohesin. The cohesin encircles DNA by forming a tripartite ring. Linkage is established between replicated DNA by an unknown mechanism. During the transition from metaphase to anaphase the Scc1 subunit of cohesin is cleaved by separase and the sister chromatids are released (A- loading of cohesin onto DNA, B- cohesin seen around un replicated DNA, C- cohesin seen around replicated DNA & D- separation of cohesin and release of DNA).

SMC 2 and SMC4 hetero dimer forms a complex with non SMC proteins CAPD2, CAPG and CAPH (Jesserbergh, 2002). All the components of this complex are required for the chromosome condensation during mitosis and cell survival. CAP D2 and CAPG contain a degenerate repetition motifs involved in protein- protein interactions called HEAT repeats (Losada & Hirano, 2005). CAPH is a kleisin subunit that links the V shaped SMC2-4 hetero dimer (Losada & Hirano, 2005). Condensin is one of the several factors required for in vivo chromosome condensation in Drosophila melanogoster (Losada & Hirano, 2005). The condensin complex introduces positive super coiling in the presence of hydrolysable ATP. Cdk-1 dependant phosphorylation of non SMC units may also play a role in the condensation of chromosomes (Losada & Hirano, 2005). Condensin also provides proper shape and elastic nature to the chromosomes. Condensin is found along the axis of chromatids is also required for intra chromatid cohesion (Nasmythy & Haering, 2005). However still there are several questions to be answered regarding the action of condensin.

Table1. SMC protein complexes and their components

Protein complex	SMC components	Non-SMC components	Function of protein complex
Cohesin	SMC1 SMC3	Scc1/Rad21	Sister chromatid cohesion
Condensin	SMC2 SMC4	CAP D2, CAP-G	Chromosome condensation
SMC5/6 complex	SMC5 SMC6	Nse1,Nse2, Nse3 and Nse4	DNA repair
Prokaryotic SMC proteins	SMC	Scp-A, Scp-B	DNA condensation and segregation

Table1 showing various SMC protein complexes, their components and their function.

7. SMC5/6 PROTEIN COMPLEX

The SMC5/6 hetero dimer shown in figure 4, forms a functional protein complex along with at least four non SMC proteins; Nse1, Nse2, Nse3 and Nse4 (Pebernard et al., 2004;

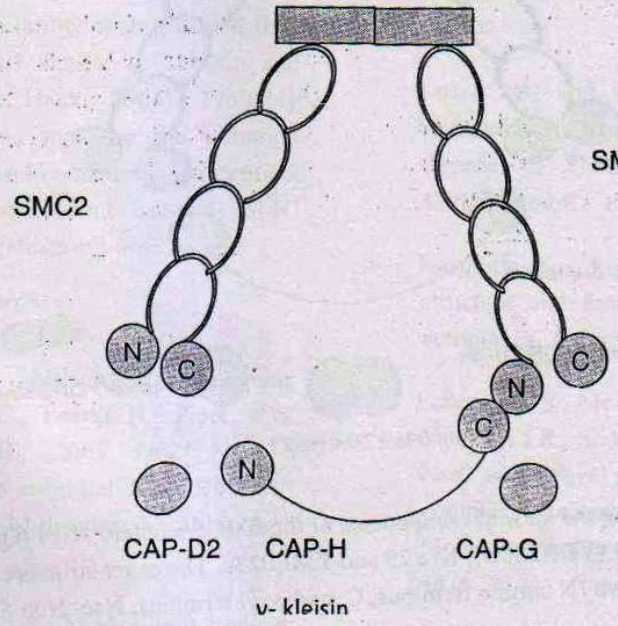

Figure5 showing the condensin complex formed by SMC2/4 hetero dimer to which CAP-H, the γ- kleisin is bound. CAP-D2 and CAP-G are Nse components of the complex (N amino terminus, C carboxyl terminus, Nse- Non SMC element).

Bin Hu et al.., 2005). In addition to these two other non SMC subunits -YML023C and Kre29 are found in Saccharomyces cerevisiae (Zhao & Blobel, 2005). The exact manner in which these subunits are associated with SMC dimer and the functional role of this complex is not clearly known. Mutant analyses indicate that all components of this complex are essential for cell cycle progression (Losada & Hirano, 2005; Bin Hu et al.., 2005). The SMC5/6 complex only binds to the replicated chromosomes, i.e. to chromosomes during S and G2 phases (Betts Lindroos et al., 2006). The complex is also recruited to DNA breaks, indicating direct function of the SMC5/6 complex in DNA repair. Even though the exact role of the SMC5/6 complex is not known, it may be inferred from the available information that this complex has a function in repair and segregation of duplicated chromosomes.

ScpA is a prokaryotic kleisin that links SMC homo dimers and Scp B binds to Scp A. This SMC-ScpA-ScpB complex pulls the replicated DNA to the opposite poles probably by DNA super coiling (Losado & Hirano, 2005). The bacterial SMC proteins have great similarities with SMC1-4 than SMC5&6 and functions similar to cohesin and condensin (Cobbe & Heck, 2004). Probably bacterial SMC proteins may be the common ancestors of cohesins and condensins. To confirm this and to know more about bacterial SMC proteins further investigations are necessary.9.

REFERENCES

1. Bin Hu, Liao, C., Milson,S.H., Mollapour, M., Prodromou, C., Pearl,L.H., Piper, P.W. and Panaretou, B., ,2005. Qri2/ Nse4 a component of the essential Smc 5/6 DNA repair complex. Molecular Microbiology; 55(6), 1735-1750

2. Cobbe, N. and Heck, M.M., 2000 Review: SMCs in the world of chromosome biology-from prokaryotes to higher eukaryotes. J.Struct Biol.; 129(2-3):123-43.

3. Hiraga,S., 2000. Dynamic localization of bacterial and plasmid chromosomes. Annu. Rev. Gennet. 34:21-59.

4. Jessberger, R., 2002. The many functions of SMC proteins in chromosome dymamics. Molecular Cell Biology, Vol.3 767-778.

5. Lee JY, Orr-Weaver TL., 2001. The molecular basis of sister-chromatid cohesion. Annu Rev. Cell Dev Biol. 17:753-77.

6. Lindroos,H.B., Strom,L., Itoh, T., Shirahige, K., and Sjogren, C., selected for publication in May 2006. The genome wide distribution of the SMC5/6 complex reveals a dual function in chromosome segregation and repair.

7. Losada, A. and Hirano, T., 2005. Dynamic molecular linkers of the genome: the first decade of SMC proteins. Genes Dev. 19(11):1269-87. Review.

8. Nasmyth, K., & Haering, C.H., 2003. The structure and function of SMC and Kleisin complexes. Annu. Rev. Biochem. 74:595-648

9. Pebernard. S., Mc Donald. W.H., Pavlova. Y., Yates III.J.R., and Boddy, N.M., 2004. Nse1, Nse2 and a novel subunit of the Smc 5- Smc6 complex, Nse3 play a crucial role in meiosis. Molecular biology of the cell. Vol. 15, 4866-4876.

10. Uhlmann,F., 2001. Secured cutting: controlling separase at the metaphase to anaphase transition. EMBO reports 2, 6, 487492

11. Uhlmann, F., 2004. The mechanism of sister chromatid cohesion. Experimental Cell Research 296; 80 85.

12. Zhao,X. and Blobel,G. 2005 A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc. Natl. Acad. Sci. 102(13):4777 82.