RESEARCH PAPER Asian Journal of Bio Science, Vol. 4 Issue 2 : 235-240 (October, 2009 to March, 2010)

Effect of novel heterocyclic compound on fertility in male albino rats

M. ANIL KUMAR[1], M. KRISHNA REDDY[1] AND M. SARANGAPANI[2]
[1]Reproductive Physiology Unit, Kakatiya University, WARANGAL (A.P.) INDIA
[2]Department of Pharmaceutical Sciences, Kakatiya University, WARANGAL (A.P.) INDIA

(Accepted : August, 2009)

The present study has been proposed to evaluate the antifertility activity of novel hetercyclic compound in male albino rats. The ethyl 1-(5-chloro-2-oxoindolin-3-ylideneamino)-1,2,3,6-tetrahydro-4-methyl-2-oxo-6-phenyl pyrimidine -5-carboxylate compound has been found to possess significant antifertility effect in rats. Intraperitonial treatment of this compound (3mg/kg b.wt/day) for 21 days did not cause any effect on body weight. The weights of testis, cauda epididymis, seminal vesicles and ventral prostate were reduced significantly. Sperm suspension was obtained from cauda epididymis to calculate the sperm count, sperm motility sperm abnormality and fertility rate. There was a significant decrease in sperm count, sperm motility, fertility rate and a significant increase in sperm abnormality. The histological picture of testis is almost normal but the number of sperms were decreased and clumping of sperms were observed in the epididymal lumen and epithelial lining also shows degenerative changes.

Key words : Heterocyclic compound, Gonadosomatic index, Fertility, Cauda epididymis

INTRODUCTION

Population explosion is a global burning problem, which affects the health and economy of world. There is an increasing need for new means of population control throughout the world. The idea of population control as a governmental or societal-level regulation of population growth does not require fertility control in the sense that it has been defined above since a state can affect the growth of a society's population even if that society practices little fertility control. To control the population explosion, investigations are undertaken to discover an effective safe and low cost contraceptive methods.

The synthetic agents available today for fertility control produce severe side effects such as hormonal imbalance, hypertension and increased risk of cancer and weight gain (Rice Wray, 1971). Thus there is a need to replace these agents by safe and effective agents such as heterocyclic compounds as contraceptive agents. Due to existing and over whelming growth rate of world population, oral contraceptive has become need of the time. But steroids have various side effects (Absar *et al.*, 2006). This creates interest to review the existing options of heterocyclic compounds having antifertility activity. However, many modern medicines are developed through the clues obtained from heterocyclic compounds. More over the heterocyclic products even today are important resources for medicine and are becoming more popular.

The chemistry of heterocyclic lies at the heart of drug discovery (Tempest, 2005). Many known active compounds contain heterocyclic cores which are indispensable elements for bioactivity (Houghteen *et al.*, 2000). The development of new fertility regulating drug from heterocyclic compounds is an attractive proposition because heterocyclics are widely utilized compounds in both pharmaceutical and agricultural fields (Lang and Lin-I, 1984). Consequently the development of methodologies useful for the assembly of molecules containing heterocyclic templates continues to attract the attention of both the academic and industrial communities (Rajanarendar *et al.*, 2008). Many heterocyclic compounds have been used as antifertility agents.

The new heterocyclic compound developed in the medicinal chemistry laboratories, University College of Pharmaceutical Sciences, Kakatiya University, Warangal has been selected for this study. This compound is prepared adopting the appropriate methods available in literature and is characterized by spectral data. The new compound possessing pyrimidine moiety because of structural similarities with nucleic acid bases exhibit various biological activities. Literature reveals that indole derivatives exhibit antifertility activity and aldose reductase inhibitory activity along with other biological activities. Keeping in view of biological significance of indole moiety and pyrimidine moiety present in the new compound, it is planed to study the effect of new compound ethyl 1-(5-chloro-2-oxoindolin-3-ylideneamino)-1,2,3,6-tetrahydro-4-methyl-2-oxo-6-phenyl pyrimidine -5-carboxylate on fertility activity adopting standard protocols available in literature.

MATERIALS AND METHODS

Animals:

Wistar stain proven fertile male albino rats weighing about 180-230 g were housed in polypropylene cages under controlled conditions (25°± 2°C and 12hr protoperiod) and were provided standard pellet food (Agro corporation Pvt. Ltd., Bangalore, India) and tap water ad libitum. Experimental procedures were adopted as approved by the animal experimentation ethics committee and maintained in accordance with the guidelines of the National Institute of Nutrition (NIN), Tarnaka, Hyderabad, India. The animals were used for the study after fifteen days of acclimatization.

Experimental design:

16 proven fertility male albino rats (weighing about 180-230gms) were divided into two groups (8 rats in each) as follows.

Group I: Rats served as control and they were intraperitoneally administered with 0.3ml of vehicle (1% sodium carboxy methyl cellulose) for 21 days.

Group II: Compound was intraperitoneally administered at the dose of 50mg/kg body wt., once daily for 21 days.

Evaluation of antifertility:

On the 22nd day, the rats were weighed and sacrificed. Testis and accessory reproductive organs *i.e.* epididymis, seminal vesicles and ventral prostate were removed cleared off fats, connective tissues and weighed on an electronic balance. The cauda epididymis was finely cut perfectly teased in 20 ml of normal saline for sperm collection. Epididymal sperm counts were made using the haemocytometer and were expressed as millions/ml of suspension. One drop of normal saline was taken on to haemocytometer and the sperm count was recorded under high power microscope.

The fertility test was performed during last five days of the treatment. The male rats were cohabited with pro-estrous females in the ratio of 1:2, respectively. The presence of vaginal plug and sperm in the vaginal smear in the next morning were considered the index for positive mating. The mated females were separated and allowed to deliver after full term.

Gonado somatic index (GSI):

The weight of testis was recorded to calculate the gonadosomatic index (GSI). The body weight of the rats were taken before sacrificed and the male reproductive organs were removed and blotted for free of blood and mucus. The testis was weighed accurately using single Panelectrical balance (Sortorias, West Germany). The gonadosomatic indices were calculated by using the formula.

$$\text{GSI value} = \frac{\text{weight of the testis}}{\text{weight of the body}} \times 100$$

Statistical analysis data were expressed as mean ± SEM and the significance of difference were analyzed by the student "t" test.

RESULTS AND DISCUSSION

The results obtained on the effect of ethyl 1-(5-chloro-2-oxoindolin-3-ylideneamino)-1,2,3,6-tetrahydro-4-methyl-2-oxo-6-phenyl pyrimidine -5-carboxylate on fertility in male albino rats are shown in Tables 1, 2 and 3. The data revealed that the body weights of rats were not much altered after the treatment. However, in the treated group a general decrease in the reproductive organ weights was observed in relation to the control. A significant reduction was observed in weights of testis epididymis, seminal vesicle and ventral prostate in treated animals. The GSI value was decreased in treated rats (Table 1).

The sperm count was significantly reduced in treated rats. The average number of sperms in control rats were 54.63 millions/ml while it was 40.29 millions/ml in treated rats (Table 2).

The result indicate that the compound has effect on the sperm morphology (Table 2). The average number of abnormal sperms per 1ml of normal saline in treated rats were 69.71% while it was 31.16% in control group.

The sperm motility of treated rats showed that the sperms were sluggishly motile without any forward progression. The motility per cent after treatment 24.80% declined significantly (Table 2).

The treated animals exhibited normal libido and mating behaviour, however, in treated animals the fertility test gave 56.25% negative fertility rate (Table 3).

The histological study of testis was carried out to evaluate the antifertility affect of this compound. In treated rats, there was a significant reduction in sperm count (Plate 1). The clumping of spermatozoa was observed in the lumen of seminiferious tubules and decrease in the lumen size of seminiferous tubules was also observed (Plate 2-3).

From the data present in Table 1, it is seen that the antifertiity activity increase in treated rats. The result of this present study revealed that the compound could cause reproductive impairment in male rats. In the present

Table 1: Effect of heterocyclic compound on body weight, accessory sex glands weight and GSI value

Sr. No	Group		Average body weight (gm)		Average organ weight mg/100g. b. wt.				GSI values
			Before treatment	After treatment	Testis	Cauda epididymis	Ventral prostate	Seminal vesicles	
1.	Control group	Mean	211.45	238.34	1239.35	521.49	387.18	576.67	1.239
		S.D.±	± 10.89	± 29.09	± 29.73	± 21.04	± 15.11	± 44.27	± 0.092
2.	Treated group	Mean	207.22	235.47	1114.71	458.35	315.64	509.89	1.115
		SD	± 21.56	± 18.27	± 41.53	± 14.63	± 19.46	± 28.31	± 0.061
		PC			(-10.05)	(-12.10)	(-18.47)	(-11.58)	(-10.01)

All the values are mean ± SD of eight individual observations
PC – per cent change over control
SD – standard deviation.

Table 2: Effect of heterocyclic compound on sperm count, sperm motility and sperm morphology in rats

Treatment		Sperm count (millions/ml)	Motility (%)	Sperm morphology (%)	
				Normal (N)	Abnormal (AB)
Control group	Mean	54.63	74.83	68.84	31.16
	SD	± 21.27	± 12.37	± 09.85	± 10.45
Treated group	Mean	35.11	44.17	30.29	69.71
	SD	± 10.64	± 26.14	± 13.38	± 7.59
	PC	(-35.73)	(-40.97)		

All the values are mean ± SD of eight individual observations.
PC – per cent change over control
SD – standard deviation.

Table 3: Effect of heterocyclic compound on fertility test in male albino rats (n=8 rats for each group)

Treatment	No. of females cohabited with males 2 females/male	No. of females showed positive mating	Fertility %
Control group	16	16	100
Treated group	16	07	43.75

investigation no significant changes were observed in body weights of animals indicating that the compound has no side effect and maintained normal general metabolism (Table 1).

The most significant change was observed in the weight of testis (Table 1). The weight of testis was reduced in treated rats. The reduction in the weight of the testis after the treatment in the present study related to the loss of spermatozoa and spermatids which make up a substantial proportion of testicular volume, by the same taken, as a consequence of disruption of spermatogenesis (Gupta *et al.*, 2004 and Dobhal *et al.*, 2000). The loss of testis weight is a result of loss of germ cells (Mathur *et al.*, 2003).

In the present investigation significant changes observed in weights of accessory sex glands (Table 1). The weights of cauda epididymis, ventral prostate and seminal vesicle were decreased. The reduction in weights of accessory sex glands and GSI, indicates that the atrophy of glandular tissue and a reduction in secretary ability (Mathur and Malarvizhi, 1995) and also it reflects an interference with androgen out put (Chaudhary and Singh, 2006). Anti androgens are known to depress the uptake of testosterone in the prostate and reduce binding of androgen to the hormonal receptors by process of competitive inhibition as reported by Sharma and Jacob (1996).

The quantity and quality of the sperms are determined by the sperm count. Cytotoxic drugs depress seprmatogenesis in mammales (Wyrobek *et al.*, 1983). A significant decrease in sperm count was noted in the epididymal suspension of treated rats. The mean sperm number was reduced 35.73% in treated rats (Table 2). Reduction in number of spermatogenic cells may be due to reduction in testicular weight and insufficient amount of testosterone (Nwanjo *et al.*, 2007.)

The percentage frequency of different abnomalities induced is shown in Table 2. The abnormalities of the epididymal sperm morphology observed were too long tail, folded, double head, ballon shaped head forms. The abnormal sperms were increased (69.71%) in treated rats. In several species abnormal sperms fail to reach the oviduct even if their proportion in the uterus is high (Krzanowska., 1974). Abnormal sperms can penetrate oocyte and develop normally (Burruel *et al.*, 1996). The abnormal spermatogenesis is caused by a shortage of androgen due to the chemical action of compound.

The data in Table 2 showed the effect of the

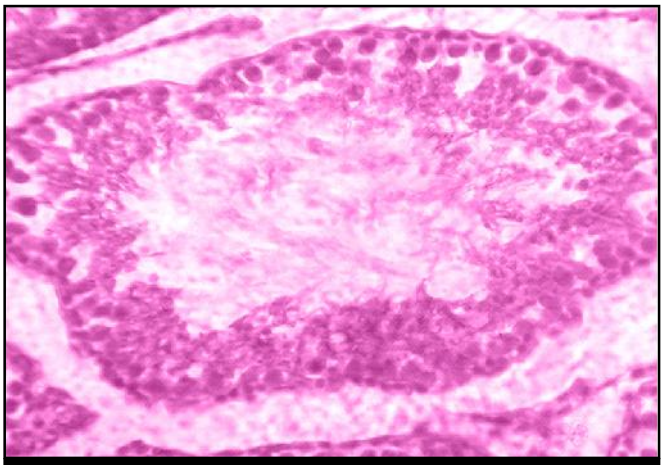

Plate 1 : Microphotograph of T.S. of testis of control rat showing seminiferous tubules consisting Spermatogonia (Sg), Spermatids (Sm) and Sperms (Sp) at higher Magnification (H x E) 40X

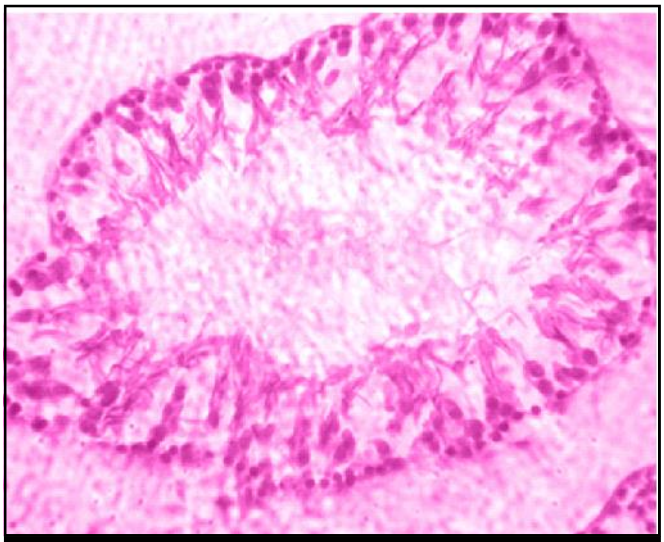

Plate 2 : T.S. of testis of treated rat showing fewer number of sperms (H x E) 40X

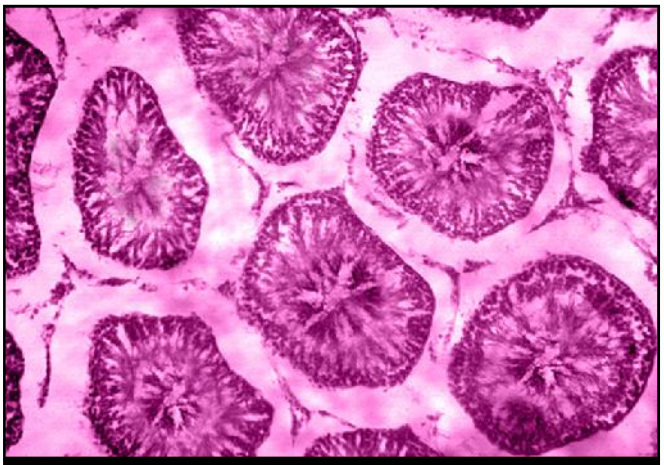

Plate 3 : T.S. of testis of treated rat showing clumping of spermatozoa in the lumen of seminiferous tubules (H x E) 10X

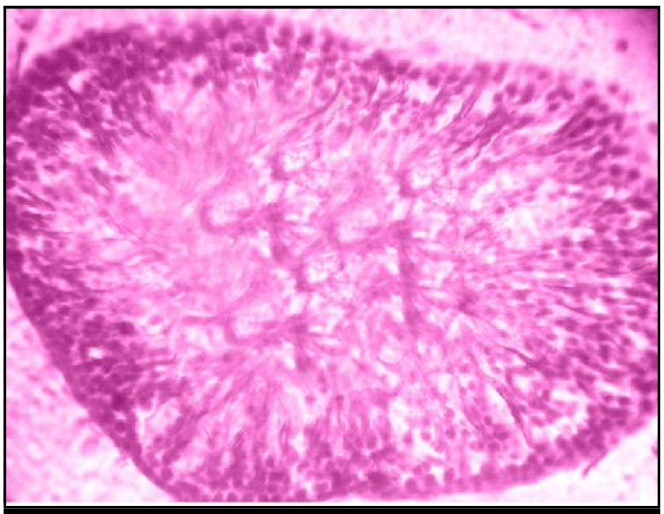

Plate 3a : Same as at higher magnification (HxE) 40X

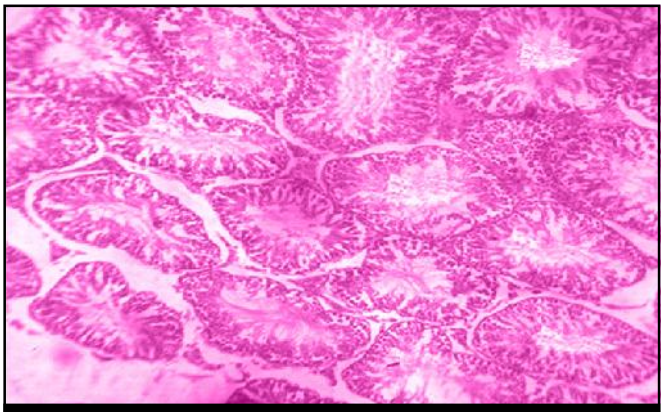

Plate 4 : T.S. of testis of compound treated rat showing more decreased in the lumen of seminiferous tubules (HxE) 10X.

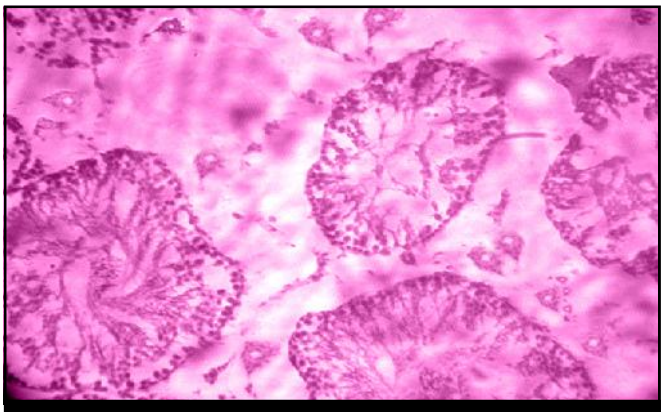

Plate 5 : T.S. of testis of compound treated rats showing mild necrotic changes in seminiferous tubules and interstitial cells (H x E) 10X.

compound on sperm motility. It was observed that the compound caused 44.17% reduction in sperm motility. The significant reduction in the sperm motility of the rats suggest that the compound was able to permeate the blood testis barriers. Rao *et al*. (1996) has reported declined sperm motility resulting in the decreased fertility.

The data in Table 3 showed the reproductive impairment caused by the compound. During the present course of the study 37.50 % negative fertility rate was observed after treatment. The reduction in sperm number is associated with reduced fertility (Mathur *et al*., 2005). The histological study of testis was carried out the antifertility effect of this compound.

Testis of treated rats showed histological changes in testicular tissue but presence of few normal seminiferious tubules together with damaged once was a common feature in testicular architecture. In the present study necrosis of seminiferous tubules, clumping of spermatozoa, reduced lumen of seminiferous tubules and reduction of sperm count were reported indicating that this compound caused a mild effect on the spermatogenic cycle and on the sperm production (Plates 1-5). Reduction in concentration of spermatozoa in epididymal lumen could be the cause of decrease level as luminal fluid of epididymis contain number of proteins (Gupta *et al.*, 2007). Similar results had been obtained in rats treated by Lupeol acetate (Gupta *et al.*, 2005).

Conclusion:

It is, therefore, concluding that this compound induced testicular damage at the tested doses and duration. The reduction in sperm count, motility and the adverse changes in the cyto-architecture of the germ cells may negatively impact on fertility in the male rats. The dearrangement of the sperm cell morphology found in this study may have implication for fertility and fetal defects. These observations suggest that the effects are probably due to an androgen deficiency caused by the anti androgens property of this compound. Thus, it is likely that reproductive toxicological risk would occur with the doses of this compound. However, this extrapolation should be made with caution, since the real human risk cannot be assessed on the basis of the present study. So further studies are necessary with human models to find out the effects of this compound as antifertility agent.

REFERENCES

Absar, A. Qureshi, Dhirendra, B. Sanghai and Padgilwar, S.S. (2006). Herbal options for contraception. A Review; Pharmacognosy magazine. 2.

Burruel, V.R., Yanagimachi, R. and Whitten, W.K. (1996). Normal mice develop from oocytes injected with spermatozoa with grossly misshapen Heads. *Biol. Reprod*., **55** : 709-714.

Chaudhary, Ashu and Singh , Ran Vir (2006). Fertility regulation in male rats by implemented tetraazamacro cyclic compounds of Iron (II). Synthetic, Spectroscopic and applied aspects with toxicological screening. *Bioinorg. Chem. Appl.,* **10** : 1155/BCA/17316.

Dobhal, M.P., Shabbir, A.S., Pranvir, P. and Gupta, R.S. (2000). Biologically active constituents isolated from medicinal plants. *Himalayan Chemical and Pharmaceutical Bulletin.,* **17** : 22-26.

Gupta, R.S., Bhatnager, A.K., Joshi, Y.C., Sharma, R. and Sharma, A. (2004). Suppression of fertility in male albino rats following a-amyrin acetate administration. *Pharmaceutical Biology*, **42**(2) : 98-104.

Gupta, R.S., Bhatnagar, A.K., Joshi, Y.C., Sharma, M.C., Khushalani Veena and Kachhawa, J.B.S. (2005). Induction of antifertility with Lupeol acetate in male albino rats. *Pharmacol.*, **75**(2) : 57-62.

Gupta, R.S., Kanwar, M. and Kachhawa, J.B.S. (2007). Effect of Methanol seed extract of Strychnos potatorum on accessory sex organs of male albino rats. *Pharmacologyonline*, **1** : 79-83.

Houghteen, R.A., Wilson, D.B. and Pinilla (2000). Use of angiopoitein like and angiopoitein like 2 nucleic acids and proteins for the treatment of blood related disorders and defects in vasculature. *Drug Discov. Today*, **5** : 276-285.

Krzanowska, H. (1974) . The passage of abnormal spermatozoa through the uterotubal junction of the mouse. *J. Reprod Fertil.*, **38** : 81-90.

Lang, S. and Lin-I, Y. (1984). In comprehensive heterocyclic chemistry. (A R Katrizky, C W Rees, Eds. Peragamon Press, Oxford) 6, 1-130.

Mathur, P. P. and Malarvizhi, D. (1995). Effect of Cisplatin on physiological status of normal rat testis. *Indian J. Exp. Biol.,* **33** (1995) 281-283.

Mathur, Neera, Mehta, Meenal and Chaudhary, Vinceta (2005). Sperm abnormality induction by food colour Metanil yellow; *J. Ecophysiol. Occup. Hlth.,* **5** : 157-160.

Nwanjo, H.U., Iroagba, I. I., Nnatuanya, I. N. and Eze, N.A. (2007). Antifertility activity of Dihydroartemisinin in male albino rats. *The Internet J.Endocrinology*, **4** (1) : 1-7.

Prasad, M.R.N., Chinoy, N.J. and Kadam, K.M. (1972). Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiological conditions. *Ferti Steril,* **23** : 186-190.

Rajanarendar, E., Siva Rami Reddy, A. and Srinivas, M. (2008). Expeditious methods for the synthesis of novel 2,3,5,6-tetrahydrobenzo(g) (1,3,5) oxadiazocine-4-thiones linked with isoxazoles., *Chinese chemical letters*, **19**(12) : 1415 – 1418.

Rao, M.V., Thakor, F., Shiney, G., Shah, K.D. and Roy, G.K. (1996). Contraceptive efficacy of testosterone enanthate in males. *J. Animal Morphology and Physiology*, **43** (1): 15-21.

Rice Wray, E. (1971). Side effects associated with steroids antifertility agents. Contraception, (Popline document number. 7105133), 137-154.

Sharma, N .and Jacob, D. (1996). Fertility suppression of male mouse after administration of Mint leaf extract. *Phytotherapy Research*, **10**(2): 175-177.

Tempest, P.A. (2005). Recent advances in heterocyclic generation using he efficient ugi multiple-component condensation reaction. *Curr. Opin Drug Discov*, **8** : 776-788.

Wyrobeck, A.J., Gordon, L.A., Burkhart, J.G., Francis, M.W., Kapp, R.W. Jr. and Litz, G. (1983). An evaluation of mouse sperm morphology test and other sperm tests in nonhuman mammals. *Mutat. Res.*, **115** : 1-72.

Wyrobek, A.J. and Bruce, W.R. (1987). Chemical induction of sperm abnormalities in mice. *Proc. Nat Acad. Sci., USA, Cell Biol.*, **72** : 4425-4429.