CHAPTER VII

CONCLUSIONS
The present investigation seeks to explain some of the factors which regulate \( \beta \)-carotene production in *Blakeslea trispora*.

The nutritional status of the culture has a strong influence on the production of carotenoids in *B. trispora* (Chapter IV). Derepression of carotene synthesis on mating was inhibited in medium containing high concentrations of glucose but not in the one where glucose was replaced with glycerol. The glucose effect was reversed by cAMP. Similarly, production of trisporic acid during mating was severely inhibited in glucose-medium and poor carbon source like glycerol enhanced both trisporic acid and carotene levels. These data indicated that regulation of carotene and trisporic acid synthesis in mated cultures may be under catabolite repression. This was further evidenced and supported by the detection of cAMP, its increased level in mated cultures and the reversal of glucose inhibition by cAMP. In this regard, \( \beta \)-carotene production follows the pattern of most secondary metabolites which are formed in post-logarithmic phase of growth and are under catabolic repression (311-313).
It has been shown that changes in cAMP levels induce an effect on growth. In *P. blakesleeanus* sporangiophores, cAMP content dropped to 50% of the initial on light stimulation before the appearance of growth response (292). Another example was provided by the dimorphic *M. racemosus* where, high cAMP levels induced yeast like growth but a significant decrease in cAMP concentration was observed during the conversion to hyphal form (290).

The observation of morphological changes induced by cAMP in plus and minus strains was similar to that induced by trisporic acid in the mating zone on agar medium. The effect of cAMP was mimicked by phosphodiesterase inhibitors.

Wolf and Mirocha (291) reported that the formation of zeaealenone, the sex hormone of *Gibberella zeae* was induced by cAMP. It is likely that some fungal sex hormones are under a repression control and that their synthesis might be induced by cAMP. In *Phytophthora capsici*, the initiation of zoosporangial formation is under the control of catabolite repression that is mediated by cAMP (308). In amoebae and in Myxomycetes, cAMP induces cell differentiation processes (309,310). This exemplifies what is very probably a general mechanism in cell systems.
of many kinds, viz. the role of cAMP in effecting a switch from vegetative to non-vegetative functions in response to nutritional circumstances. In above mentioned system there are no data to indicate whether cAMP affects gene transcription rather directly, as in the control of catabolite repression in bacteria (287).

Other than nutritional control, some intracellular proteins appear to control carotenogenesis in single and mated cultures. Presence of a repressor which might not allow excess carotene synthesis has been hypothesized (199). Our data indicate that unmated plus and minus strains contain proteinic factors which inhibit carotene production. During mating the regulation over carotene pathway is disturbed and this observation has been well documented in *E. trispora* (222, 294). Formation of trisporic acid during mating is responsible for the upsurge in carotene biosynthesis. Therefore it was thought that TA acts as a derepressor of carotene pathway. We have observed that exogenous supplementation of TA to minus cultures resulted in the inactivation of inhibitory proteinic factors (Chapter VI).

A conspicuous increase in proteolytic activity on mating or on trisporic acid supplementation to minus cultures suggests that proteases may be associated with
trisporic acid functions. They could well inactivate the IP and thereby stimulate carotene synthesis. This is further evidenced by our observation of the presence of a stimulatory fraction (SP) in cell-free extracts of mated cultures. Proteolytic activity co-purified with SP activity and it is probable that the SP is a specific protease. However, further purification of SP is required before a definite identity of SP can be established.

Biological activities of IP and SP were studied by adding them to intact cells of minus strain. Therefore their action on the carotene synthesizing system will have to be explained either by the entry of these proteins into the cells or alternatively, interaction with some surface receptors which might trigger off some specific reactions. Both possibilities are equally probable. Export and import of proteins through membranes is known. Many extracellular enzymes such as amylase, protease, pectinase, cellulase, asparaginase etc. have been isolated from the culture media whereas some proteins were shown to enter the cells for e.g. colicins in E. coli (314). However, more experimentation is required before the molecular mechanism of the above mentioned proteinic factors in carotene biosynthesis can be explained.
The stimulation of carotene production by the hormone trisporic acid may be culmination of many possible reactions as has been proposed schematically in Fig.1.

Even when plus and minus mycelia are kept apart without substrate contact, mating response was still found and thereby it was suggested that volatile metabolites from the opposite mating types can induce trisporic acid formation (266). It seems now that these volatile compounds may have a very important role in triggering the series of reactions resulting in zygospore formation and increased carotene levels. More work should be done to understand the nature and significance of these metabolites. They may be mainly responsible for trisporic acid formation.

Trisporic acid seems to exert influence at various steps in the cell. It could be increasing cAMP levels possibly by activating adenyl cyclase. No information is available to support involvement of cGMP in any of above processes. However, we have included this possibility in the scheme as it could induce morphological changes in B. trispora minus.

On mating, increased uptake of inorganic ions such as phosphorous and magnesium was observed (268). This might have provided more co.factors for carotene pathway.
Volatile compounds or and cAMP

**HORMONE TRISPORIC ACID**

- Change in amounts of c-AMP/c-GMP (?)
- Permeability to ions, inorganic P, Mg++
- Increased proteolytic activity for new turnover of proteins
- Derepression at molecular level rRNA, mRNA new proteins

**Metabolic effects**

**More supply of co-factors**

**CAROTENE**

**Enzymes, stimulators**

**Fig. 1. Scheme proposing regulation of carotenogenesis in Blakeslea trispora**
Increased proteolytic activity and change in protein patterns in presence of trisporic acid indicate that turnover of intracellular proteins may be resulting in formation of proteins which facilitate more carotene production. Trisporic acid could also act at the transcriptional level, as its stimulatory effect is associated not with activation of pre-existing carotenogenic enzymes, but with their synthesis in de novo (206,315).

The activation of carotene biosynthetic pathway was not restricted to trisporic acid but also to many naturally available compounds such as abscisic acid (ABA), β-ionone, α-ionone and vitamin A which share significant structural similarity with trisporic acid. They all could stimulate carotene pathway as discussed in Chapter III. The magnitude of stimulatory activities of these effectors were in the order as follows: trisporic acid > ABA > β-ionone > α-ionone > vitamin A. Comparison of structures and stimulatory activities of all the effectors indicated that length of the side chain and presence of a keto group in the ring structure of TA molecule contributed significantly for the biological activity to carotenogenesis. Stimulations mediated by TA, β-ionone, ABA and vitamin A were cycloheximide sensitive indicating that new protein
synthesis was required. Trisporic acid and \( \beta \)-ionone mediated stimulations were competitive in nature. Presence of TA/\( \beta \)-ionone in higher amounts interfered with the stimulatory activity of \( \beta \)-ionone/TA. Data suggested that they probably competed for the same site in carotene biosynthetic pathway. It was proposed that trisporic acid, like \( \beta \)-ionone, might be derepressing the initial steps in carotene synthesis which catalysed \( C_5 \) unit formation from mevalonate phosphate. Precursors of trisporic acid, abscisic acid and vitamin A (316) were carotenoids. \( \beta \)-Carotene served as a precursor of trisporic acid and vitamin A (57-59,254,286). Many reports have appeared in recent times indicating that ABA is formed by a photooxidation of epoxy carotenoids (304,305). ABA is a recently discovered growth-regulating substance which promotes senescence and abscission of leaves and induces dormancy in buds and seeds (306).

ABA has been detected in many carotenogenic systems such as tomatoes, rose petals, avocado etc. And it is generally noticed that amounts of carotene change depending upon the growth stage of that particular system. A possible involvement of ABA in triggering off high carotene synthesis in these systems is implicated. Similarly, it was found that various citrus oils
enhanced carotene production in *B. trispora* (307). This was attributed to the presence of terpenic compounds. Thus all the information available regarding the TA and its structural analogues suggest that they might be stimulating carotenogenesis apart from other functions in the systems they are found.

It was earlier reported that β-ionone, vitamin A and abscisic acid could not induce zygophore formation in *M. muceda* (243). This clearly indicated that the other functions of trisporic acid could not be mimicked by these compounds. A possible explanation for this could be that the mating phenomenon induced by trisporic acid is a complex reaction and requires the interaction of other factors. One of these could well be cAMP as we observe that cAMP induced morphological changes are similar to those induced by trisporic acid.