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Integration of the Metabolic Pathways of Steroids, Carotenoids, and Retinoids

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CONTENTS

I. INTRODUCTION

The biosynthetic pathway for the formation of isoprenoid compounds in plants is based on the pathway of sterol biosynthesis studied for many years in animals and yeast. In plants, isoprenoid biosynthesis may be seen as a central pathway from acetyl-CoA via mevalonate and isopentyl pyrophosphate to long chain prenyl pyrophosphates, with many branch points leading to the individual isoprenoid compounds.

II. EVOLUTION OF STEROIDS

It appears that cell function is influenced by steroids, even in the first signs of life on earth. The biological action of steroids in many plant species appears comparable to that in animals.[1] Although some ancestors left no fossil record, steroids have been constantly present during evolution not only as cell constituents but also as bioregulators.[2,3] In fact, the present-day pattern of steroid secretion from specialized glands appears to be relatively recent in evolutionary terms.[2,3]

A number of bacteria, fungi, and photosynthetic vascular plants synthesize steroids that are hormonally active in animals. The cellular effect of such steroids in microbes and plants appears, by and large, to be comparable to that in mammals. Evidence suggests that steroid action in botanical phyla is mediated via receptors organized in a manner similar to that seen in animals.[1] Therefore, it is more helpful to emphasize the similarity in cell ultrastructure (e.g., cell function and biochemistry; the induction, synthesis and function of enzymes; the receptors, respiration, and the structure and function of membranes) rather than to emphasize the differences.

III. SIMILARITY OF PLANT AND ANIMAL ISOPRENOID BIOSYNTHESIS

In plants, isoprenoid synthesis may be described as a main pathway from acetyl-CoA, via mevalonate and isopentenyl pyrophosphate, to long chain prenyl pyrophosphates, with many branches leading to individual isoprenoid compounds. This is shown schematically in Figure 1, where some of the main isoprenoid compounds found in plants are included.[4] Isopentenyl pyrophosphate is important as a precursor of isoprenoid compounds, and the metabolic organization of the pathways using this intermediate is an important factor in the regulation of isoprenoid metabolism in plants. Knowledge of the metabolic pathways and the enzymes is needed for understanding of the regulation of isoprenoid metabolism, particularly of any reactions that might divert intermediates away for isoprenoid biosynthesis.

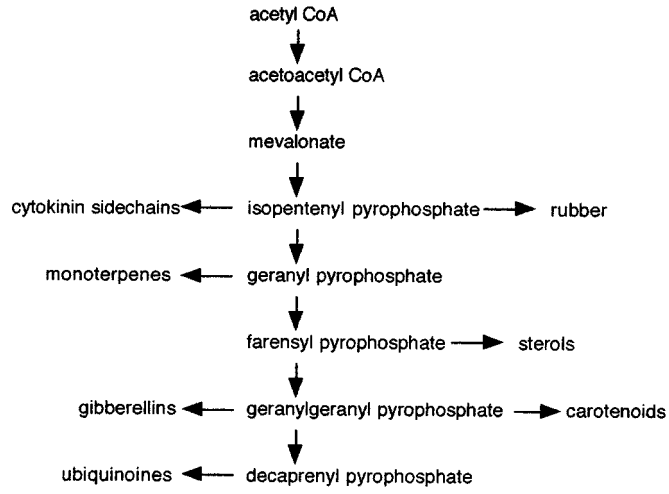

Figure 1 Wide range of isoprenoids found in plants.

The corpus luteum is a unique source of steroids, carotenoids and retinoids in mammals. The bovine corpus luteum develops during every normal estrous cycle (average 20 days). The corpus luteum is a small gland (4.1 to 7.4 g) that develops rapidly (maximum. size in 7 to 8 days) from the ovulated follicle.[5] It performs vital functions in the reproductive process, such as secretion of progesterone, which is necessary for implantation of the blastocyst. After implantation, the continued secretion of progesterone is needed to maintain a quiescent uterus and an intrauterine environment conducive to continued development of the embryo. Not all of the functions of the corpus luteum have yet been discovered.

IV. PORTER-LINCOLN METABOLIC PATHWAY IN THE BOVINE

The first mammalian biosynthesis of β-carotene from acetate was with bovine corpus luteum tissue.[6] This indicated that the Porter-Lincon metabolic pathway was also to be found in mammals. Therefore, the synthesis from acetate to farnesyl pyrophosphate is the same for sterols and β-carotene. Then farnesyl pyrophosphate and isopentenyl pyrophosphate form geranylgeranyl pyrophosphate. The condensation of two molecules of geranylgeranyl pyrophosphate leads to phytoene, to phytofluene and eventually to β-carotene, Figure 2. β-Carotene can then be metabolized to retinol.[7] Retinol is also important for the synthesis of cholesterol and the metabolism of various steroids, such as pregnenolone and progesterone.

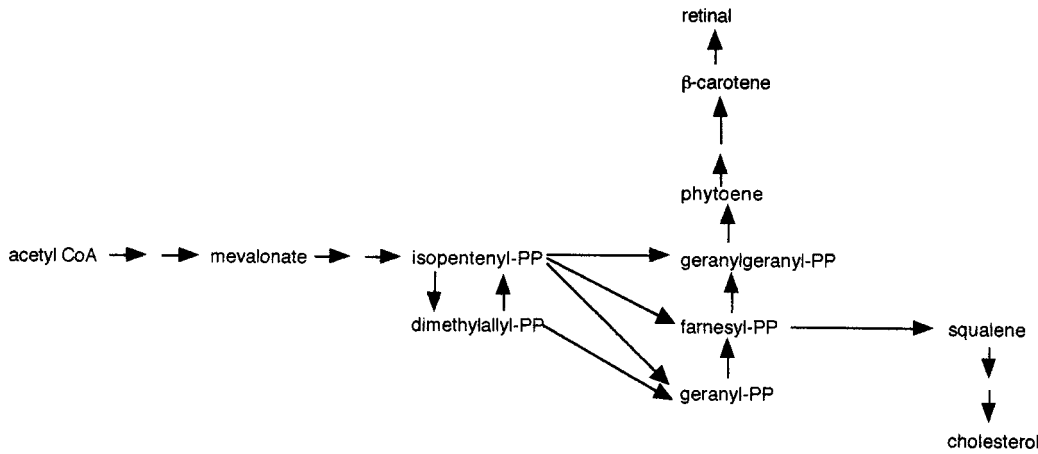

Figure 2 Isoprenoid metabolic pathway to steroids, carotenoids, and retinoids.

V. BETA-CAROTENE BIOSYNTHESIS IN THE OVARY

Ovarian carotenoids were reported in 1913[8] and again in 1930.[9] The bovine corpus luteum contains relatively high concentrations of β-carotene amounting to 60 μg/g of tissue weight.[6] Retinal has also been isolated from this tissue.[7] Corpus luteum tissue, when sliced and incubated with β-[15,15′-^{3}H]carotene, yields radioactive retinal[10] and oxidized carotenes. This indicates that the ovary possesses the enzymes to synthesize retinal *in situ*, which have a role in reproductive functions. At ovulation time, specific activity of the carotene cleavage enzymes was two times greater in the ovary than in the intestine.[11]

A. VITAMIN A AND STEROID PRODUCTION

Vitamin A was suggested as a possible requirement for efficient steroid hormone production. For instance, Das et al.[12] reported that the mean concentrations of serum retinol and serum cholesterol of ovarian cancer patients in Singapore were significantly lower than in noncancerous control subjects. There were moderate direct correlations between retinol and cholesterol levels in the control subjects, but not in the cancer patients. Retinoids in vertebrates represent an essential class of nutrients needed for the maintenance of differentiated epithelial structures, vision, reproductive functions, and overall health. Retinoic acid, for example, has multiple effects on cell growth and differentiation, and it appears to be organogenic in embryogenesis.[13]

Several retinol-binding proteins (RBP) related to but distinct from plasma are secreted by the rat uterus. It has been suggested that RBP may carry retinol to the placenta. It has also been demonstrated that placental membranes of many species produce RBP. The conceptus RBP is probably identical to plasma RBP. Since vitamin A is known to play a critical role in embryo development, the many RBP-like molecules produced by the conceptus and the surrounding tissue probably ensure that an optimal amount of retinol is delivered to the embryo.[14]

The research by Gloor and Wiss[15] provided the clue for our β-carotene biosynthesis study with the bovine corpus luteum. Gloor and Wiss[15] demonstrated that ^{14}C-labeled mevalonic acid is incorporated into the ubiquinone (coenzyme Q) molecule by the rat liver. The rate of incorporation was found to depend on the nutritional supply of vitamin A. An increase in the rate of incorporation could be found with a greater depletion of vitamin A. They also discovered strong indications that cholesterol and squalene synthesis is influenced by vitamin A deficiency.

Gloor and Wiss[15] reported that vitamin A depletion had an influence on ubiquinone, cholesterol, and squalene synthesis. Cholesterol synthesis was reduced from 94% to 45%; squalene synthesis increased from 2% to 31%; and that of ubiquinone from 2% to 14%. They found mevalonic acid incorporation into squalene increased continuously during the vitamin A depletion period. The incorporation of mevalonic acid into ubiquinone reached a plateau after two weeks.

After giving a single dose of vitamin A to animals whose vitamin A levels had been depleted for three and a half weeks, they studied whether vitamin A acted at the metabolic level. Twenty hours later mevalonic acid incorporation into cholesterol had risen from 44% to 75%; that into squalene had decreased from 31% to 14%. The ubiquinone synthesis was only slightly changed. These values corresponded quite well with those obtained after one week of vitamin A depletion. The early biochemical alterations, observed after such a short deficiency period, and their rapid reversion after a single dose of vitamin A, suggest an action of vitamin A on a metabolic level. It is assumed that either vitamin A or a metabolic derivative is involved in one of the last steps of cholesterol synthesis. Squalene (and other precursors) and its metabolic derivative are presumed to be enriched in the vitamin A deficient state.

By utilizing the published research of Gloor and Wiss,[15] we were able to prove that the bovine corpus luteum can synthesize β-carotene from acetate. This was done by Barry Austern, a former graduate student in my laboratory. He used radioactive acetate and bovine corpus luteum slices in media to synthesize the C_{40} molecule.

The results of the research[6] are given below.

1. Acetate-^{14}C was incorporated into β-carotene by slices of corpus luteum tissue. Tissue slices also incorporated mevalonic acid into β-carotene. Homogenates of this tissue did not incorporate radioactivity into β-carotene. Anaerobic nitrogen atmosphere gave the best yields.
2. Maximum incorporation of acetate into β-carotene was achieved with the addition of vanadyl sulfate, an inhibitor of squalene cyclase. The incorporation reached a maximum after a three hour incubation.
3. Diphenylamine reduced the synthesis of β-carotene to 35% of control. Diphenylamine has been shown to block the conversion of phytofluene to other carotenoids in the Porter-Lincon pathway.

4. NAD stimulated the reaction.
5. Only corpus luteum tissue metabolized radioactive acetate to β-carotene. Bovine liver, adrenal cortex, ovary stroma, and also guinea pig liver did not metabolize acetate to β-carotene.
6. Proof that the radioactive material was β-carotene came from the identification of the material by thin-layer chromatography, and by visible and infrared spectroscopy. The material was crystallized to constant specific activity on two separate occasions.
7. With the use of antibiotics, other animal tissues, and differing preparations, it was demonstrated that β-carotene was synthesized by the corpus luteum and not by bacterial contamination.

Farnsworth et al.[16] labeled HeLa cells with [^{3}H]mevalonic acid and unequivocally demonstrated that an all-trans geranylgeranyl group was a major isoprenoid modification. It was noted that the proteins that are modified with a geranylgeranyl group and the prenyltransferase that attaches the isoprenoid group to proteins remain to be identified; geranylgeranyl pyrophosphate is assumed as a substrate in the reaction. The geranylgeranyl pyrophosphate synthetase that synthesized this isoprenoid polyphosphate from isopentenyl pyrophosphate and farnesyl pyrophosphate has been purified from pig liver.[17]

VI. RETINOID ACTION ON PROGESTERONE METABOLISM

Piziak and Gawienowski[18] found that progesterone-4-^{14}C can be metabolized by guinea pig placenta tissue to 20α-hydroxyprogesterone to a significant extent in an atmosphere of 95% oxygen:5% carbon dioxide. Retinol increased the metabolism of this reaction by tenfold.

Vitamin A deficiency is known to have an adverse effect on the size of the ovary and on the integrity of the uterus and would thus have an effect on reproductive ability.[19] But a more direct effect on steroid metabolism probably exists. One clue that suggests this fact is the disappearance of vitamin A from the ovary after menopause.[20] Ganguly et al. discovered that vitamin A deficient rats secreted less than normal amounts of progesterone and 20α-hydroxyprogesterone.[21] Juneja et al. reported a decrease in conversion of Δ^5-3β-hydroxysteroids into Δ^4-3-ketosteroids in tissues that were only mildly deficient in vitamin A.[22] Retinal also stimulated the metabolism of progesterone to pregnenolone[23] (Figure 3).

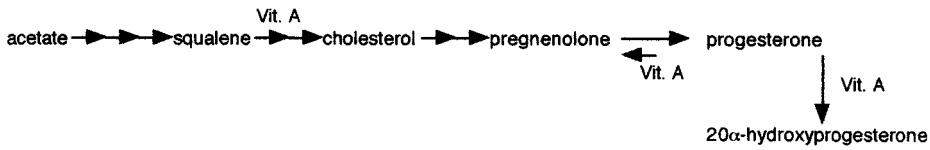

Figure 3 Vitamin A role in progesterone metabolism.

There is evidence that retinoids can regulate the expression of both progesterone and its receptors in appropriate target cells. In granulosa cells, treatment with rather high levels of retinol or retinoic acid (10^{-6} *M*) resulted in substantial increases in progesterone accumulation in the presence of substrates for steroidogenesis.[24] Conversely, in the human breast cancer cell line T-47D, whose growth is markedly inhibited by retinoic acid, treatment with retinoic acid (ED_{50}, 10^{-8} *M*) resulted in a marked concentration-dependent decrease in the level of progesterone receptor mRNA protein, and binding.[25] The effect on progesterone receptor mRNA was rapid, being detectable at 1 hour and maximal by 6 hours after the addition of retinoic acid. Further studies showed that retinoic acid acted directly to inhibit transcription of the receptor in a transient fashion. The decreased expression of progesterone receptors resulted in impaired responsiveness to progestins, suggesting that retinoids might modulate the effects of steroid hormones in breast cancer and reduce the sensitivity of breast cancers to progestin therapy.[26]

VII. SUMMARY

In summary the biosynthetic pathway for the formation of isoprenoid compounds in plants is similar to the pathway of sterol biosynthesis in animals and yeast. In plants, isoprenoids are synthesized from acetyl-CoA via mevalonate and isopentyl pyrophosphate to long-chain prenyl pyrophosphates. These compounds may then be metabolized to form various isoprenoid compounds. The bovine corpus luteum, which utilizes the Porter-Lincon pathway, has been shown to metabolize acetate to steroids and β-carotene. For example, farnesyl pyrophosphate is an intermediate in the production of steroids and β-car-

otene. The synthesis of carotenoids continues with the condensation of two molecules of geranylgeranyl pyrophosphate to phytoene (C_{40} hydrocarbon), phytofluene, and eventually to β-carotene.[27] β-Carotene can then be metabolized to retinol. Retinol is important for the synthesis of cholesterol and the metabolism of various steroids, such as pregnenolone and progesterone. The action of vitamin A on progesterone metabolism is important for embryonic development.

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NOTES ADDED IN PROOF

Carotenoids are tetraterpenoids synthesized by nuclear-encoded multienzyme complexes located in the plastids of higher plants. Norris et al.[28] demonstrated conclusively that plastoquinone is an essential component of phytoene desaturation in Arabidopsis. Whether ubiquinones are involved in the desaturation of phytoene in the bovine corpus luteum remains to be determined.

Nadzan[29] reviewed the highlights of the recent advances in molecular and cellular biology of retinoid receptors and retinoids that have led to intense interest in these regulators of gene transcription. He also summarizes the most current evidence supporting the role of retinoids in cancer chemotherapy.

An unusual role for isoprenyl intermediates was discovered in the research of a protein that suggests involvement in human cancers and association with membranes through a covalently bound isoprenyl lipid. The protein, Ras protein, is a product of the ras gene, a mutant version of a normal gene that encodes a GTP-binding protein. The normal protein and a number of related GTP-binding proteins are known to act in signal transductions activated by growth factors, hormones, neurotransmitters and other signals.[30] The mutant ras gene is found in many humans with cancers, and the mutant gene product is believed to be responsible for the uncontrolled division of cancerous cells. Many believe that inhibitors of protein prenylation could be effective therapeutic agents for the treatment of some human cancers.[30,31]

Farnesyl and geranylgeranyl isoprenoids are the main two of the covalently bound isoprenyl lipids studied. They are also two main intermediates of β-carotene (Figures 1 and 2). One can wonder whether this is only a coincidence.

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