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# Biological functions of carotenoids – diversity and evolution

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**Abstract.** Carotenoids first emerged in archaebacteria as lipids reinforcing cell membranes. To serve this function their long molecules have extremely rigid backbone due to the linear chain of usually 10 to 11 conjugated C=C bonds in *trans*-configuration – the length corresponding the thickness of hydrophobic zone of membrane which they penetrate as "molecular rivets". Carotenoids retain their membrane-reinforcing function in some fungi and animals.

The general structure of carotenoid molecule, originally having evolved for mechanical functions in membranes, possess a number of other properties that were later used for independent functions. The most striking fact is that these properties proved to fit some new functions to perfection.

– The polyene chain of 9–11 double bonds absorbs light precisely in the gap of chlorophyll absorption – function as accessory light-harvesting pigments in all plants;

– Unique arrangement of electronic levels owing to the by polyene chain structure makes carotenoids the only natural compounds capable of excitation energy transfer both (i) from carotenoid excited state to chlorophyll in the light-harvesting complex and (ii) from triplet chlorophyll or singlet oxygen to carotenoid in photosynthetic reaction centers – protection of RC from photodamage.

The linear system of conjugated C=C bonds provides high reducing potential of carotenoid molecules making them potent antioxidants in lipid formations. Still, there is a lack of evidence of the chemical antioxidant function of carotenoids, especially in higher organisms; most data demonstrate an antioxidant ability rather than a function.

Carotenoids have many other independent biological functions, including: specific coloration patterns in plants and animals, screening from excessive light and spectral filtering, defense of egg proteins from proteases in some invertebrates; the direct carotenoid derivative – retinal – acts as visual pigment in all animals and as chromophore in bacteriorhodopsin photosynthesis, retinoic acid in animals and abscisic acid in plants serve as hormones. All these functions utilize various properties (mechanical, electronic, stereospecific) of a single structure evolved in bacteria for a single membrane-reinforcing function, thus demonstrating an example of pure evolutionary preadaptation.

One of the practical conclusions that can be reached by reviewing uniquely diverse properties and functions of carotenoids is that, when considering possible mechanisms of their effects in organisms (e.g., anticarcinogenic action), all their functional traits should be taken into account.

## 1. Introduction

Carotenoids are the most ubiquitous and widespread natural pigments, they are characteristic for organisms of all major taxa. Purpleness of purple bacteria, brownness of brown algae, pink flesh of salmon and yellow stain of mussels, black carapace of crayfish, a variety of bird feather or reptile skin colours

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Table 1

| Structure | Polyene chain | | | | |
|---|---|---|---|---|---|
| Properties | electronic | | | mechanic | stereospecific |
| | ability for close-range energy transfer | Π-electron cloud mobility | | rigidity | |
| | | light absorption | high reducing potential (easily oxidized molecule) | | |
| Functions | transfer of absorbed light energy to chlorophyll (all photosynthetics) | photosynthetic antenna pigments (all photosynthetics) | antioxidant defense via elimination of free radicals (bacteria, plants, animals) | stabilization of membrane fluidity (bacteria, mycoplasms, funghi, mollusca) | animal (retinoic acid) and plant (abscisic acid) hormones |
| | | light reception in vision (retinal in all animals) | | | |
| | triplet chlorophyll quenching (all photosynthetics) | coloration – species /sex/social status specific (animals) – masking (animals) – attractive or repellent (animals, plants) | defense against $NO_2$ nitrosative action (plants) | stabilization and defense of carotenoproteins in skeleton structures (crustaceans, molluska, echinoderms) | |
| | singlet oxygen quenching ($O_2$-photosynthetics) | chromophor in bacteriorhodopsin photosynthesis (*Halobacterium*) | | defense of reserve proteins in molluscan and crustacean eggs against proteases | |
| | | light-protective screening (shown in green microalgae) | | | |
| | | selective light filtering in retina (insects, birds) | | | |
| | | metabolic activation via heating (lake planktonic crustaceans) | | | |

– these are the most notable examples of carotenoid presence. Being so widely distributed, carotenoids have a great number of independent biological functions – a unique one among biocompounds (Table 1).

## 2. Mechanical functions

Carotenoids first emerged in archaebacteria. They are, may be, the first natural pigments. Their first function in the oldest of Earth organisms was that of lipids reinforcing bacterial cell membrane. To serve this function their long molecules have an extremely rigid backbone due to the linear chain of usually 10 to 11 conjugated C=C bonds – the length corresponding to the thickness of the hydrophobic zone of the membrane that they penetrate as “molecular rivets” [12]. Their polyene structure is very hard to bend or twist.

Being incorporated in membrane, carotenoids decrease its fluidity, so their amount controls the stability of that important parameter (liable to change with temperature) affecting all membrane functions. Carotenoids retain their membrane reinforcing function in mycoplasms, some fungi and animals. This is why the flesh of many mollusca and ascidians has yellow-to-red colour [9,10,14,16].

Mechanical properties of carotenoids were used at later stages of evolution for protection of certain proteins:

- Complexes of carotenoids and proteins (carotenoproteins) in exposed outer structures, e.g., crustacean cuticle, molluscan shells, echinoderm skin, are highly resistant to environmental action due to reciprocal defense of carotenoid and protein parts [20].
- The major reserve proteins in molluscan and crustacean eggs are carotenoproteins; although many proteases present in cytoplasm, reserve carotenoprotein is not affected by them, because carotenoids defend the protein core from digestion. When cell division starts, the complex dissociates and the protein part is used for developmental needs [2,6].

The general structure of the carotenoid molecule, originally evolved for purely mechanical functions in membranes, possesses a number of other properties that were later used for independent functions (already in archaebacteria the oxidized half of a carotenoid molecule, retinal, was recruited as a chromophor in bacteriorhodopsin photosynthesis of *Halobacterium halobium*). The most striking fact is that other properties of carotenoids proved to fit some new functions to perfection.

## 3. Photosynthetic and antioxidant functions

The polyene chain of 9–11 double bonds absorbs light in the gap of chlorophyll absorption (420–500 nm), which enables carotenoids to function as accessory light-harvesting pigments in all plants, thus increasing the active spectral range of photosynthesis [15].

A polyene length of 9 conjugated bonds is shown to be the minimal one for quenching singlet oxygen and effective elimination of dangerous radical species. The long Π-electron system is very mobile and provides high reducing potential of carotenoids – they are easily oxidized. Being incorporated in membranes or dissolved in other lipid formations, they protect those structure from oxidation by aggressive radical species; carotenoids themselves are irreversibly destroyed in this process [11]. This is shown to be the case, for example, in developing embryos of sea urchin [17].

The unique arrangement of molecular energy levels provided by polyene makes carotenoids the only natural compounds capable of close-range excitation energy transfer [3]:

- from the carotenoid excited state (S1) to chlorophyll S1 in the light-harvesting complex, this is how light energy caught by carotenoid is channeled to photosynthetic reactions;
- from the triplet state of chlorophyll (a highly unstable state produced in photosynthesis and resulting in destruction of that molecule) to triplet carotenoid;
- from the singlet state of oxygen (an extremely destructive species produced in intensive photosynthesis), to carotenoid triplet state.

The two latter processes take place in photosynthetic reaction centers of all photosynthetics. They are absolutely vital for protection of RC from photodamage – carotenoidless mutants can live only in the very dim light. Carotenoid return from triplet to ground state just dissipats the excessive energy as heat.

Those events of energy transfer between very short-living excited states are possibly due to the very close range interactions inside the pigment-protein complex (RC or LHC).

## 4. Vision

Carotenoids provid the base for visual reception – the oxidized halves of several carotenoids, i.e. the retinals, are receptor molecules in eyes of all animals. Retinals, complexed with the protein opsin, isomerize upon absorbing light quants, causing change of conformation of the whole rhodopsin, thus launching the further cascade of reactions leading to nerve excitation. This process very much resembles the light reception in ancient bacteriorhodopsin photosynthesis. Retinal, complexed with three different types of opsin, can absorb light of different wavelengths – this is the base for colour vision in mammals.

## 5. Communicative coloration

Light-absorption within visible range is used for the most spectacular function of carotenoids – communicative species-specific coloration of plants and animals. This is:

- Species and sex specific coloration for the animals of the same species to recognize each other; the truly bright examples are found in birds the great majority of them being colored by carotenoids;
- Sex/social status coloration; some coral fish change their color pattern (due to carotenoids) as a sign of changed sex and social status;
- Masking coloration; a wonderful example is the chameleon;
- Attractive coloration; many yellow and orange flowers and fruits;
- Warning coloration; coral aspid, for example.

Coloration of plants in many cases is due to antocyans, in animals primarily due to melanins, although really bright animal coloration is always the work of carotenoids.

Two other interesting ways of the use of carotenoid pigmentation are:

- Green microalga *Haematococcus pluvialis* accumulates large amounts of the carotenoid astaxanthin in cytoplasm under high light. The function of this pigmentation is shown to be the screening of chloroplasts from excessive light.
- The red cuticle of planktonic copepodes in temperate lakes serves for rising body temperature. The carotenoids absorb solar radiation and dissipate the energy as heat thus activating crustacean metabolism [1].

## 6. Stereospecific functions

Some direct derivatives of carotenoids act as hormones. Retinoic acid (oxidized half of beta-carotene molecule) in mammals is a hormone regulating epidermal growth [13], abscisic acid (modified part of higher plant carotenoid neoxanthin) in plants is one of the key growth hormones [7]. These functions are neither based on the rigidity of carotenoid molecules, nor on their unique electronic properties. The configuration of molecule *per se* is valuable, fitting as a key to the receptor lock.

There are also cases of unknown functions of these pigments, e.g., in mammalian ovary, function of light dependent esterification of siphonoxanthin in some algae [19], or functions of unknown mechanisms like the regulation of photosynthetic yield by carotenoids' interconversions (xanthophyll cycle) in light-harvesting complex [5,8].

## 7. Evolutionary considerations

Over 600 carotenoids are known to date. The main differences between them concern end groups that are needed mostly for binding to, or recognition by proteins, and orientation in membranes. The biggest alteration carotenoid molecule undergoes for acquiring a new function is the oxidative cleavage resulting in the retinoid structure. The main structural and functional feature of carotenoid molecule – polyene chain – is retained in all species of the family.

The overview of the diversity of carotenoids' biological functions shows that the general structure of the carotenoid molecule, originally evolved for membrane-reinforcement in archaebacteria, remained practically unchanged in the great diversity of organisms and functions. Carotenoids proved to be tailor-made for many roles, some of which are of vital importance – like their protective function in photosynthetic reaction centers. The fact that they are very ancient and conserved natural pigments suggests that, to some extent at least, carotenoids determined the shape and the manner of operation of the new structures; this may apply to photosynthetic and visual systems.

One of the practical conclusions that can be reached by reviewing the uniquely diverse properties and functions of carotenoids is that, when considering possible mechanisms of their effects in organisms, all their functional traits should be accounted for. Thus, current research of the mechanisms of the anticarcinogenic action of carotenoids tends to concentrate on their pre-vitamin and antioxidant functions, whereas the membrane-modulating power of these pigments exceeds that of the anticancer drug tamoxifen [4,18].

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