Myxococcus xanthus

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Myxobacteria are Gram-negative δ-proteobacteria found predominantly in terrestrial habitats and often brightly colored due to the biosynthesis of carotenoids. Carotenoids are lipophilic isoprenoid pigments that protect cells from damage and death by quenching highly reactive and toxic oxidative species, like singlet oxygen, generated upon growth under light. The model myxobacterium *Myxococcus xanthus* turns from yellow in the dark to red upon exposure to light because of the photoinduction of carotenoid biosynthesis. How light is sensed and transduced to bring about regulated carotenogenesis in order to combat photooxidative stress has been extensively investigated in *M. xanthus* using genetic, biochemical and high-resolution structural methods. These studies have unearthed new paradigms in bacterial light sensing, signal transduction and gene regulation, and have led to the discovery of prototypical members of widely distributed protein families with novel functions. Major advances have been made in elucidating the molecular mechanisms underlying the light-dependent signaling and regulation of the transcriptional response leading to carotenogenesis in *M. xanthus*.

Keywords: photoreceptor ; photosensitizer ; photoregulation ; Myxococcus xanthus ; plasmalogen ; singlet oxygen ; carotenoids ; transcription ; lipids ; optogenetics

1. Overview

Light is an important and ubiquitous signal in terrestrial and aquatic ecosystems, and the ability to sense, respond and adapt to light is crucial for most living organisms, including bacteria. Photosynthetic bacteria capture and convert light, an essential energy source, to chemical energy for cellular utilization, but light is also important for several other cellular processes and responses such as phototaxis, development, virulence, circadian rhythms and UV-induced DNA damage repair. However, light can be harmful and cause cell damage and death because excitation of photosensitizing biomolecules, such as porphyrins, chlorophyll or flavins, generates highly reactive oxygen species (ROS) like singlet oxygen ($^1O_2$), superoxides, peroxides and hydroxyl radicals that can destroy cellular DNA, protein and lipid components. Consequently, bacteria have evolved ingenious mechanisms and machineries to mount a protective response to counter photooxidative stress [1].

A commonly used defense mechanism against photooxidative damage is through the biosynthesis of carotenoids, which quench and dissipate as heat the excess energy of $^1O_2$ and other ROS produced upon illumination. Carotenoids constitute a major class of lipophilic isoprenoid derivatives that are characterized by an extended, typically all-*trans*, conjugated polyene chain (usually C_{40} and some C_{50}, C_{45} and C_{30} terpenes) with acyclic, monocyclic or bicyclic ends (their oxygenated derivatives are called xanthophylls). Most are richly colored (light yellow to deep red), since they absorb blue-violet light (400–500 nm range) and fulfill biological roles besides photoprotection, such as in photosynthetic light harvesting, signaling and as precursors of photosensory molecules and hormones. All photosynthetic organisms (plants, algae or bacteria) and many non-photosynthetic fungi, archaea and bacteria synthesize carotenoids, whereas animals, save some strikingly few exceptions, do not but obtain them exogenously. The principal environmental factors involved in signaling and triggering carotenoid biosynthesis are light and oxygen-related species like $^1O_2$. in plants, fungi and bacteria.

Light-induced carotenogenesis and its regulation in the Gram-negative soil bacterium *M. xanthus* is undoubtedly one of the best studied and characterized among bacteria. Considerable progress has been achieved on the mechanistic, structural and photochemical aspects of light-regulated carotenogenesis in *M. xanthus*. The work has uncovered new and large protein families, such as an entirely new class of photoreceptors with a novel mode of action and revealed the participation of “eukaryotic-like” proteins, including one that is absent in bacteria except for *M. xanthus* and related myxobacteria, but which occurs in animals and turned out to be a long-sought human enzyme conserved across metazoa.

Two mechanisms operate to sense light and trigger carotenogenesis in *M. xanthus*. Direct light-sensing and gene regulation by the B_{12}-based CarH photoreceptor controls transcription by modulation of CarH oligomeric state and thereby

DNA binding by 5'-deoxyadenosylcobalamin (AdoCbl) or coenzyme B_{12}, a biological form of vitamin B_{12}, and light. Here, AdoCbl binds to apo form monomers (or molten globule tetramers) to produce active, properly folded, compact tetramers that bind to DNA and block transcription in the dark. UV, blue or green light photolyzes CarH-bound AdoCbl and disrupts DNA-bound tetramers to monomers (or dimers) that retain photolyzed AdoCbl with loss of operator binding leading to transcription.

In the second mechanism, light is perceived through photoexcitation of protoporphyrin IX, a hydrophobic cyclic tetrapyrrole that is the immediate precursor of heme in its biosynthesis. This leads to production of $^1O_2$, which together with a special kind of lipid called plasmalogen, signals a complex genetic pathway starting with the inactivation, by a still unknown mechanism, of a membrane-bound negative regulator called CarR, which liberates a specific factor CarQ belonging to the ECF-σ family of factors required to initiate transcription of defined genes. CarQ, in association with RNA polymerase and some additional protein factors (CarD, CarG, IHF), initiates transcription of one carotenoid synthesis gene and a gene at another locus that produces an antirepressor CarS. The latter leads to derepression of a large cluster of nine other carotenoid synthesis genes. Thus, light ultimately triggers expression of the ten genes that encodes enzymes leading to carotenoid synthesis in *M. xanthus*.

2. Conclusions and implications

Delving into how *M. xanthus* "sees" and mounts a photooxidative stress response that triggers carotenogenesis uncovered two novel pathways and with them new paradigms in bacterial light sensing, signal transduction and gene regulation, as well as the discovery of prototypical members of widely distributed protein families with novel functions. One pathway relies on a form of vitamin B_{12} and its association with a single photoreceptor-cum-transcriptional factor, and the other is a B_{12}-independent, more complex route that requires various singular factors. Many worthy firsts can be credited to elucidation of the two pathways. This includes discovery of one of the first ECF-σ factors, CarQ; the founding members of the large protein family of B_{12}-based CarH photoreceptors that occur in bacteria; the founding members of the CarD_CdnL family of bacterial RNA polymerase-binding transcription factors that are widely distributed in bacteria and occur in *M. xanthus* and other δ-proteobacteria, α-proteobacteria, Actinomycetes, Firmicutes, Deinococcus-Thermus and Spirochaetes, but not in β-, γ- or ε-proteobacteria, Chlamydiae or Cyanobacteria; the long-sought human desaturase involved in plasmalogen biosynthesis through its *M. xanthus* homolog. Insights specific to *M. xanthus* and closely related bacteria, but also ones more broadly conserved across bacteria, have emerged. This photooxidative stress response is linked, directly or indirectly, to responses to copper, to heme and fatty acid biosynthesis, and shares global regulators with processes as diverse as fruiting body development and activation of CRISPR-Cas systems. Future work will undoubtedly reveal new, possibly surprising, interconnections to other cellular activities.

Beyond bacterial physiology, signaling and gene regulation, the findings from *M. xanthus* light-induced carotenogenesis have had other important ramifications. How this response and its unique factors are conserved across bacteria and other organisms provides valuable evolutionary insights, especially since it involves some factors that are more typical of eukaryotes than other bacteria. Indeed, one hypothesis for the origin of eukaryotes known as the Syntrophy hypothesis posits that an ancestral early myxobacterial-like deltaproteobacterium may have participated in the symbiosis or syntrophy that produced the first eukaryotic cell because of the many eukaryotic-like genes in myxobacteria like *M. xanthus*. For example, the study of *M. xanthus* light-induced carotenogenesis has led to the discovery of a human/animal lipid desaturases essential in plasmalogen synthesis. This not only revealed a remarkable conservation of this enzyme across a vast evolutionary distance, but also has important implications in human health and disease, since plasmalogens have been linked to various human disorders including cancer and Alhzeimer´s disease. Knowing the identity of this long-sought desaturase helps to directly assess the role of plasmalogens in diverse pathologies, and has already proved useful in studies of mitochondrial metabolism and ferroptosis. Satisfyingly, CarH has now been exploited as one of the few green-light responsive optogenetic tools for light-controlled gene expression in *M. xanthus* and transgene expression in mammalian and plant cells; in receptor interactions and signaling in human cells and zebra fish embryos; in the generation of protein hydrogels for facile encapsulation and release of cells and proteins, and in cell adhesions that have been adapted to address challenges in regenerative neurobiology for sustained delivery of neuroprotective cytokines aimed at neuronal survival and axon regeneration *in vivo*.

References

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