

## DISCOVERY AND EVOLUTION OF GFP

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The green fluorescent protein GFP and its derivatives are widely used as the markers of proteins, cells and tissues, and now considered to be indispensable tools in biological and medical research. The usefulness of GFPs stems from the fact that the fluorescent proteins have a built-in chromophore in their primary structures, so that the proteins can be cloned and expressed in live organisms. We discovered GFP in 1961 as a trace contaminant during our first large-scale extraction and purification of the  $\text{Ca}^{2+}$ -sensitive photoprotein aequorin from the luminescent jellyfish *Aequorea aequorea*. Thus, the discovery was incidental. Moreover, the fluorescent protein discovered was of no importance at all at the time except for its role as the light-emitter of the jellyfish. Considering that GFP exists in an extremely small amount in *Aequorea* and certain other coelenterates, the fluorescent protein was probably not discovered if a large-scale aequorin work had not been carried out.

Our study on aequorin was initiated in consequence of a series of fortuitous events and unanticipated happenings that occurred during the 15-year period before the discovery of aequorin, as if the entire sequence in that period had been programmed for the discoveries of aequorin and GFP by the Mother Nature. The large-scale work of aequorin enabled us to accumulate a sufficient amount of GFP to study its properties. The chemical structure of GFP chromophore was determined in 1979, showing the uniqueness and importance of the protein. Helped by the developments in genetic engineering, GFP was cloned in 1992, and then expressed in live organisms by Chalfie in 1994, triggering the explosive popularity of GFP. Various modified forms of GFP were genetically produced by Roger Tsien and others. Red fluorescent proteins of the GFP family were found in corals in 1999. Recently, far-red fluorescent proteins were obtained from a sea anemone. The improvements and applications of the GFP-family fluorescent proteins still continue.