INTRODUCTION

It is a fundamental feature of the organisation of eukaryotic cells that they contain organelles possessing genetic systems additional to the one located in the nucleus. In the case of chloroplasts, the fact that these organelles contain both DNA and ribosomes was demonstrated first in 1962. It soon became apparent that both these components are present in significant quantities. Thus the chloroplast genome has the potential capacity for encoding about 125 proteins, each of molecular weight 50,000, whilst chloroplast ribosomes can represent up to 50% of the total ribosomal complement of leaves [1,2]. The existence of such quantities of chloroplast DNA and ribosomes prompts the question as to their roles in the formation of chloroplasts. Which genes are encoded in chloroplast DNA? Which proteins are synthesised by chloroplast ribosomes? I believe it is necessary to answer such simple direct questions before it is possible to tackle meaningfully the more interesting but far more complex question as to the molecular basis of chloroplast development.

THE USE OF INTACT ISOLATED CHLOROPLASTS

When I entered this field in 1970, it was clear that little progress had been made in identifying either the genes located in chloroplast DNA, or the proteins which are synthesised by chloroplast ribosomes. The chloroplast genome occurs in multiple copies in each chloroplast. This makes conventional genetic analysis difficult, because a mutation in one copy will be swamped for a long time by all the other wild-type copies. The other question,
as to the function of chloroplast ribosomes, had been tackled by two methods. The first method was to determine the effect of treating cells making chloroplasts with antibiotic inhibitors believed to be specific for chloroplast ribosomes. The results of such experiments carried out in different laboratories were often conflicting; I believe this is partly due to the lack of specificity of action of antibiotics in some tissues [2]. The second method was to identify the products of protein synthesis by isolated chloroplasts. Preparations of isolated chloroplasts were known to incorporate labelled amino acids into proteins, but no convincing identification of any protein had been reported; most papers showed that the products tended to be polydisperse rather than discrete in nature. Nevertheless, it seemed to me in 1970, that the identification of protein and RNA molecules made by isolated chloroplasts was, in principle, the most direct and unambiguous method for determining the function of the chloroplast genetic system. If a method could be found for preparing isolated chloroplasts in which translation is coupled to transcription, and in which both processes occur with fidelity, it should be possible to determine both the structural genes present in chloroplast DNA and the function of chloroplast ribosomes. Since 1970, we have developed in my laboratory a system in which isolated chloroplasts from pea, spinach, barley and maize leaves use light energy to make discrete RNA and protein molecules, some of which we have identified. The coupling of transcription to translation however, still eludes us, so we cannot conclude that because a protein is made inside the chloroplast it is therefore encoded in chloroplast DNA.

The rationale of our approach is that in order to produce discrete, identifiable protein and RNA molecules, conditions must be used in which correct elongation, termination, and release of chains occurs. It seemed to me that such conditions were more likely to be met in intact chloroplasts, rather than in the lysed systems that were commonly used, and we therefore adopted methods that were currently in vogue for preparing intact chloroplasts capable of high rates of photosynthetic carbon dioxide fixation. It is characteristic of such intact chloroplasts that they will use light as a source of energy in the absence of added cofactors. By using light as the source of energy for protein and RNA synthesis, it is thus possible to ensure that the incorporation of labelled precursors is taking place solely in intact chloroplasts. It is therefore not necessary to use purified preparations of chloroplasts for these studies, because other organelles, as well as broken chloroplasts, cannot use light to synthesise ATP. Once the pattern of protein synthesis by intact chloroplasts had been established, it provided a base-line which enabled us both to fractionate the system, and to assess the results of in vivo inhibitor experiments. This approach has been very successful in establishing the basic patterns of protein synthesis in chloroplasts isolated from several