Compartmentation of metabolism within mitochondria and plastids

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Abstract

An important component of metabolic regulation is compartmentation and specialization. Subcellular compartmentation and the role of individual organelles is well studied, though less consideration has been given to the extent to which organelles differ between cells. Organelles extracted from whole tissue homogenates will have generally originated from a range of cell types. This review describes and assesses the regulation of metabolic activity in plants at both the cellular and subcellular level by considering specialization of mitochondria and plastids.

Key words: TCA cycle, alternative oxidase, glycine oxidation, phosphate translocators, starch, nitrogen metabolism.

Introduction

Compartmentation is an important component of metabolic regulation. Many metabolic pathways are highly compartmentalized, for example, between different subcellular organelles. In all eukaryotic cells, compartmentation of metabolism within organelles concentrates enzymes and metabolites and also separates them, as a means of controlling reactions. Although subcellular compartmentation and the role of individual organelles has been the subject of many studies in plant metabolism, it is less common for cellular compartmentation to be considered. This review will illustrate how compartmentation at both the cellular and subcellular level contributes to the regulation of metabolic activity in plants. Particular emphasis will be placed on the specialization of mitochondria and plastids. Vacuoles are described elsewhere in this issue (Miller et al., 2001).

Cellular compartmentation

An important requirement of metabolic studies is a knowledge of the pool size and rate of turnover (or flux) of metabolites. Analysis of the concentration of components of soluble extracts taken from whole tissue homogenates is often the basis for calculating the endogenous concentration of a metabolite. Although great care is usually taken to ensure the accuracy of the analyses, the endogenous concentration is calculated, more often than not, by making crude estimates of the volume of the cell compartment from which the metabolite was extracted. Errors are compounded when the metabolite is present in more than one compartment or, worse still,
in a highly specialized cell, where its local concentration could be extremely high. Single cell-sampling analysis (as described by Tomos and Sharrock, 2001) and tissue dissection methods (described by Outlaw and Zhang, 2001) offer valuable tools for investigating metabolite and enzyme compartmentation. To allow for comparisons to be made between these sampling techniques and those studies where whole tissue homogenates have been used, a detailed analysis of cellular and subcellular compartmentation was carried out in wheat leaves (Hopkins, 1997; L Hopkins and AK Tobin, unpublished results; Tables 1 to 3).

Tissue sampled from the mature region of a 7-d-old wheat primary leaf was first analysed by light microscopy (×250) to determine the volume fraction (i.e. ratio of the volume of a component to the containing volume; \( V_V \)) of the leaf occupied by each major tissue type. Stereological methods were used as described earlier (Weibel, 1969; Weibel and Bolender, 1973). The mature wheat leaf tissue was found to consist of 27% (\( V_V \)) epidermal, 51% mesophyll and 4% vascular tissue, with the remaining fraction (c. 15%) being occupied by air space (Table 1). These values are similar to those reported previously for barley (Winter et al., 1993; Table 1) and spinach (Winter et al., 1994; Table 1). Given that 70 g of fresh wheat leaf tissue would have a total volume of approximately 100 ml (including air space; Hopkins, 1997) a crude homogenate would then produce 51 ml of mesophyll, 27 ml of epidermal and 4 ml of vascular tissue extract, if all cells were extracted homogeneously. So, if a metabolite was, for example, restricted to the vascular tissue, its concentration in a crude homogenate would be c. 5% (4/(51 + 27 + 4)×100) of that in the intact tissue. The potential error in estimating metabolite concentration is therefore considerable.

This is further illustrated by measuring subcellular compartmentation of wheat leaves by stereological analysis of electron micrographs (×5000; Weibel and Bolender, 1973; Hopkins, 1997). The volume contained within the plasma membrane of a mature mesophyll cell was found to be compartmentalized into the following fractions (\( V_V \)): 9% cytosol, 22% chloroplast, 68% vacuole, and 0.6% mitochondrion (Table 2). These values are similar to those found previously for barley (Winter et al., 1993) and spinach (Winter et al., 1994; Table 2). If, as explained above, only 51% of the original leaf tissue is occupied by the mesophyll, then out of a total leaf extract, 4.6% (i.e. 9% of 51%) of its volume would consist of mesophyll cytosol, 11.4% would be mesophyll chloroplast, 34.7% mesophyll vacuole, and 0.3% mesophyll mitochondria (Table 3). The remainder would consist of air space and vascular and epidermal cells.

### Table 1. Tissue composition of mature leaves as a percentage of leaf volume (% volume fraction; \( V_V \))

<table>
<thead>
<tr>
<th>Compartiment</th>
<th>Wheat</th>
<th>Barley</th>
<th>Spinach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophyll</td>
<td>51</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td>Vascular</td>
<td>4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Epidermal</td>
<td>27</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>Air space</td>
<td>18</td>
<td>23</td>
<td>32</td>
</tr>
</tbody>
</table>

*Data from Hopkins (1997), L Hopkins and AK Tobin (unpublished results).
*Data from Winter et al. (1993).
*Data from Winter et al. (1994). NB, the data of Winter et al. (1994), also included the apoplast (4.5% \( V_V \) for barley and 5% for spinach).

Volume fractions were calculated by measuring the cross-sectional area of each tissue type present on sections taken at random from mature regions of 7-d-old wheat primary leaves, viewed under a light microscope (×250). The area of each compartment (\( A_{\text{compartment}} \)) was determined and divided by the total area of leaf tissue visible on the section (\( A_{\text{leaf}} \)) to give \( A_V = A_{\text{compartment}}/A_{\text{leaf}} \). Given that \( A_V = V_V \times V_{\text{unit}}/V_{\text{leaf}} \) (Weibel, 1969), \( V_V \) was taken directly from each \( A_V \) value. A total of 20 micrographs was analysed and these had been taken at random from tissue sampled from 20 different leaves.

### Table 2. Volume fraction (\( V_V \% \)) of a mature wheat leaf mesophyll cell occupied by major subcellular components

<table>
<thead>
<tr>
<th>Compartiment</th>
<th>Wheat</th>
<th>Barley</th>
<th>Spinach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuole</td>
<td>68</td>
<td>73</td>
<td>79</td>
</tr>
<tr>
<td>Plastids</td>
<td>22</td>
<td>19</td>
<td>9.5</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.6</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Cytosol</td>
<td>9</td>
<td>6.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*Data from Hopkins (1997), L Hopkins and AK Tobin (unpublished results).
*Data from Winter et al. (1993).
*Data from Winter et al. (1994). The value for plastid \( V_V \) in spinach is for stromal volume and not for total plastid volume as given in a and b. \( V_V \) was calculated by measuring the cross-sectional area of each subcellular compartment, divided by the total area of the space within the plasma membrane of the mesophyll cell, viewed by TEM (×5000). A total of 20 micrographs was analysed and these had been sampled at random from tissue taken from 25 different 7-d-old wheat seedlings from five independent experiments.

### Table 3. Compartmentation of mature wheat primary leaf tissue; subcellular compartment volume as a percentage of leaf volume (Hopkins, 1997; L Hopkins and AK Tobin, unpublished results)

<table>
<thead>
<tr>
<th>Compartiment</th>
<th>Mesophyll</th>
<th>Epidermal</th>
<th>Vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuole</td>
<td>34.7</td>
<td>19.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Plastid</td>
<td>11.4</td>
<td>4.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Mitochondrion</td>
<td>0.3</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Cytosol</td>
<td>4.6</td>
<td>4.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td>51.0</td>
<td>27.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Values were derived from data presented in Tables 1 and 2 as follows: the volume fraction (%) of leaf tissue occupied by each cell type (Table 1) was multiplied by the volume fraction (%) of mesophyll cell occupied by each of the individual subcellular compartments (Table 2) and divided through by 100 to give the percentage of total leaf volume occupied by each subcellular compartment. The values are an approximation, given that the subcellular compartmentation of the epidermal and vascular cells is likely to be significantly different from that calculated for the mesophyll cell (as discussed in the text).
An analysis of subcellular compartment volumes within the vascular or epidermal cells has not been completed but, by assuming that these cells are subdivided into the same fractional volumes as that found in the mesophyll, approximations can be made. This will certainly overestimate the size of the plastid fraction, because mature chloroplasts are a major component of the mesophyll cell, whereas plastids are relatively infrequent in vascular and epidermal cells (L. Hopkins and AK. Tobin, personal observations).

The major compartment of the leaf, irrespective of cell type, is clearly the vacuole (Table 3). In a total leaf homogenate, approximately 56% of its volume would be derived from the vacuoles of mesophyll (34.7%) epidermal (19.0%) and vascular (3.0%) cells. This means that if intact vacuoles were isolated from leaf tissue, assuming equivalent recoveries from each cell type, then over 61% of the vacuolar volume would consist of mesophyll cell vacuole, over 33% would be from the epidermis, and the remaining 6% would come from the vascular tissue. For a mitochondrial fraction, over 55% of mitochondria would be from the mesophyll, 37% from the epidermis and 8% from the vascular cells. The cytosolic fraction is particularly interesting because only 50% of it would have originated in the mesophyll cells (4.6/4.6 + 4.0/6) x 10 with the epidermis contributing almost the same volume, at 43%, and the remaining 7% coming from the vascular cells. Given that the subcellular volumes calculated for the epidermis most likely underestimate the cytosolic fraction, the actual proportion of epidermal cytosol is most likely greater than this. This means that a so-called ‘soluble fraction’ from leaf tissue contains at least as much cytosol from non-photosynthetic cells as it does from the photosynthetically mature mesophyll cells. This has important implications in the determination of metabolite concentrations and in the interpretation of enzyme compartmentation in photosynthetic tissue.

These examples also illustrate the potential heterogeneity of isolated organelle preparations. Organelles extracted from whole tissue homogenates are likely to have originated in a range of different cell types that will also be at various stages of development. As the examples presented below illustrate, both mitochondria and plastids show considerable variation in form and function depending on their cellular location, and on the developmental stage of the cell in which they are present. These factors must be taken into account when attempting to ‘scale up’ from in vitro studies on isolated organelles to whole tissue processes.

Subcellular compartmentation

An organelle in a particular tissue often serves a single, specific metabolic function. Perhaps the most obvious metabolic difference relates to whether a tissue is photoautotrophic or heterotrophic. Associated with this are clear and well documented differences in organelle structure and function, the most obvious being the presence of chloroplasts in photosynthetic tissue and, in contrast, of non-photosynthetic plastids in roots and other non-green tissue. In this review, examples will be presented to show that organelle specialization is more subtle than this, with evidence of heterogeneity of organelle form and function occurring within apparently similar tissue. For the purpose of this review, mitochondria and plastids will be of primary concern (vacuoles will be considered elsewhere in this issue; Miller et al., 2001).

Mitochondria: structure, function and interaction with other organelles

Structure

Mitochondria are bioenergetic organelles and, despite the variations in form and function that will be discussed later, their major role is to synthesize ATP, by coupling substrate oxidation to electron transport and the generation of a proton electrochemical gradient, or proton motive force (Δp). Mitochondria exist in a variety of shapes and sizes, but generally are 1–2 μm long by 0.25–0.5 μm in diameter. Each mitochondrion is bounded by an outer membrane that acts as a barrier to large molecules (> 10 kDa) which can only enter the mitochondria by means of specific pores located within the lipid bilayer (Newcomb, 1990). The inner mitochondrial membrane is folded into cristae that permeate the soluble, internal matrix. Components of the mitochondrial electron transport chain (ETC) are an integral part of the inner membrane. It is also the location for the ATP synthase, part of which extends into the matrix, and of succinate dehydrogenase (SDH). The enzymes involved in the TCA cycle and glycine oxidation, as well as the pools of metabolites, NAD, NADP, ADP, and ATP, are all present in the matrix.

Despite familiarity with the textbook images of mitochondria, it is important to note that their shape is continually changing within the living cell. This is becoming apparent as imaging techniques, such as confocal microscopy and GFP-tagging enable mitochondria to be observed in situ (Hanson and Köhler, 2001; Logan and Leaver, 2000). Gross differences in morphology have been known for some time in mammalian tissue. Mitochondria in heart tissue, for example, have more densely folded cristae and this is thought to be related to their high respiratory activity (Nicholls and Ferguson, 1992). There is increasing evidence of structural variation in plant mitochondria, prompting questions of whether this reflects differences in function, as discussed below.
Function

Two of the three processes of respiration, the TCA cycle and oxidative phosphorylation, occur within the mitochondrion, whilst the third, glycolysis, occurs in the cytoplasm and supplies the mitochondria with substrates such as pyruvate from the oxidation of carbohydrate. Protein and lipid degradation can also provide substrates, feeding into the TCA cycle as pyruvate, 2-oxoglutarate and oxaloacetate, from amino acid metabolism, and as acetyl CoA following β-oxidation. As well as providing reductant (NADH, FADH$_2$) for generation of Δp and ATP, the TCA cycle serves as an important source of carbon skeletons for biosynthetic processes (Douce, 1985; ap Rees, 1990). The biosynthetic function, whilst not unique to plants, is likely to be more important in the mitochondria of an autotrophic organism. Even so, the major demand on the mitochondria within photosynthetic cells is for the oxidation of glycine that is generated during photorespiration (Walls Grove et al., 1992). As photorespiration originates in the chloroplast, due to the oxygenase reaction of Rubisco, glycine oxidation is intrinsically linked with photosynthesis.

Oxidation of substrates generates reductant that has to be reoxidized in order to maintain flux. The mitochondrial ETC provides one route for reoxidation, with the subsequent generation of Δp and ATP synthesis. Plant mitochondria have the facility to regulate the extent to which the ETC is coupled to ATP synthesis by having alternative routes for electron transport. Whereas mammalian mitochondria oxidize NADH via Complex I with the concomitant production of 3 mol ATP, plant mitochondria possess an additional non-proton-pumping rotenone-resistant NADH dehydrogenase on the inner mitochondrial membrane that passes electrons directly into the Q pool. This results in synthesis of just 2 mol ATP per mole of NADH oxidized (Palmer, 1976). The presence of an additional terminal oxidase, the alternative oxidase, in plant mitochondria provides increased flexibility in metabolism. This acts as an alternative to cytochrome oxidase and, as it accepts electrons directly from the Q pool, provides a non-phosphorylating route for electron transport. Another mechanism for adjusting ATP synthesis is provided by an uncoupling protein (UcP) found initially in mitochondria of brown adipose tissue where it functions in thermogenesis (Nicholls, 1979). A similar protein has been identified in plant mitochondria (plant uncoupling mitochondrial protein, PUMP; Vercesi et al., 1995). The uncoupling effect, in mammalian mitochondria, occurs as a result of fatty acid translocation. UcP translocates the anionic form of the fatty acid to the outside of the mitochondria, while the protonated form re-enters by diffusion, thus fatty acids act as cycling protonophores (Garlid et al., 2000). PUMP is thought to operate in a similar way, as discussed below.

There are thus a number of different requirements for mitochondrial metabolism, ranging from biosynthesis to catabolic processes that may or may not result in ATP synthesis. Although it is feasible for all of these processes to occur simultaneously within the same organelle, it is equally likely that, as the metabolic demands differ between cells and tissues, so does the composition and activity of the mitochondria. There is increasing evidence that mitochondria do indeed differ in protein composition in different types of plant tissue.

Heterogeneity of mitochondrial form and function

The changes in metabolic activity that occur with changes in development of plant tissue offer a useful means of investigating the relationship between mitochondrial form and function. Studies of seed germination, leaf development and the onset of thermogenesis during flowering in Arum lilies all provide evidence of mitochondrial heterogeneity.

Changes in mitochondrial composition during seed germination have been reported for a range of species. In parallel with an observed increase in respiratory activity, changes in constituents such as DNA and phospholipids together with enzyme activities have been reported (Akazawa and Bevers, 1956; Breidenbach et al., 1967; Solomos et al., 1972; Morohashi and Bewley, 1980; Rickwood et al., 1987; Ehrenschild and Brambl, 1990). In starch-storing seeds, such as cowpea (Vigna sinensis), mung bean (Vigna radiata) and soybean (Glycine max), an increase in respiration during the early stages of germination was observed (Morohashi, 1986) despite there being only small changes in activity of the mitochondrial enzymes SDH, malate dehydrogenase (MDH) and cytochrome oxidase. In contrast, during germination of lipid-containing seeds such as pumpkin (Cucurbita sp.), cucumber (Cucumis sativus) and castor bean (Ricinus communis), increased respiratory activity coincided with marked increases in the activity of these enzymes, due primarily to de novo synthesis. There was a much greater increase in cytochrome oxidase and MDH activity than there was in SDH activity during germination of the lipid-containing seeds, indicating differential development of the mitochondria. A more detailed, and prolonged, investigation of germination in C. sativus has been carried out (Hill et al., 1992). It was found that during lipid mobilization within the developing cotyledons, the activity of the decarboxylating reactions of the TCA cycle was restricted. However, once photosynthesis developed the mitochondria acquired the capacity to carry out the complete TCA cycle. Similar results were reported in castor bean endosperm, where the activity of enzymes and oxidation of substrates on the non-decarboxylating side of the TCA cycle were higher than those on the decarboxylating side (Cooper and Bevers, 1969; Millhouse
et al., 1983). This contrasts with soybean, where no difference is observed in the capacity of different sections of the TCA cycle (Bryce and Day, 1990), perhaps reflecting fundamental differences in mitochondrial biogenesis in starch-containing as compared with lipid-containing seeds (suggested by Morohashi, 1986). Logan et al. were able to isolate two distinct populations of mitochondria from the embryos of germinating maize seeds (Logan et al., 2000). Both populations were poorly developed in the dry, pre-imbibed embryos. The heavier population (fractionated at 37–42% (w/v) sucrose) developed the capacity to oxidize a range of substrates and also showed ultrastructural development, such as formation of cristae, within days of imbibition. In contrast, the lighter population (fractionated at 22–28% (w/v) sucrose) showed no significant change in structure or activity during imbibition. The development of the heavy mitochondria was also shown to be progressive, with differential changes in the capacity to carry out specific functions. Whereas an active electron transport chain was present in both populations, TCA cycle activity was measurable only in the heavy fraction after hydration of the tissue and this increased during germination. Despite lacking TCA cycle activity, mitochondria present in dry embryos were able to generate ATP by oxidizing exogenous NADH, thus providing energy for early biogenesis. From this range of studies of mitochondrial biogenesis in germinating seeds, it would appear that the common observation is of a differential development of mitochondrial function, with the capacity for ETC, ATP synthesis, TCA cycle activity, and oxidation of NADH and glycine all changing according to the metabolic requirements of the germinating tissue.

Developing leaf tissue provides an alternative model system in which to study mitochondrial biogenesis. In this case, changes in photosynthetic activity enable comparisons to be made between mitochondria in autotrophic and heterotrophic tissue.

Leaf development is most easily studied in grasses, where cell division is confined to a basal intercalary meristem and this results in a linear gradient of increasing cell age between the base and tip of the leaf. The most notable metabolic change that occurs with leaf development is an increase in photosynthesis (Baker and Leech, 1977; Tobin et al., 1988; Thompson et al., 1998) and photorespiration (Tobin et al., 1988). The increase in photorespiration results in an increased demand for mitochondrial glycine oxidation and the activity of glycine decarboxylase (GDC), the enzyme complex that oxidizes glycine, has been found to increase 5-fold with the development of wheat leaf mesophyll cells (Tobin et al., 1988; Rogers et al., 1991). The amount of each of the GDC subunits, P, H, T, and L, was also found to increase, although the L protein showed a pattern of development that was distinct from that of the other subunits (Rogers et al., 1991). The apparent concentration of one of the subunits, the P protein, was studied in some detail using immunogold-labeling. It was found not only to increase in concentration within the mitochondria during mesophyll cell development, but also to differ in concentration between cells, with the mesophyll cell mitochondria having much higher concentrations than those of the vascular parenchyma (Tobin et al., 1989). Given that the vascular parenchyma cells contain non-photosynthetic plastids (Tobin and Yamaya, 2001) the reduced level of GDC in the mitochondria of these cells correlates with a reduced requirement for photorespiratory glycine metabolism.

Studies of C3–C4 intermediate species demonstrate an even more specific control over GDC expression. C3–C4 intermediates are characterized by having a CO2 compensation point that is intermediate between those of C3 and C4 plants (Hunt et al., 1987). This is thought to be due to a combination of anatomical and biochemical features. Mature leaves of C3–C4 intermediates, such as certain species within the genera Moricandia, Flaveria and Panicum, have an unusual arrangement of organelles within the bundle sheath cells that surround the vascular bundles. The mitochondria and peroxisomes are aligned by the part of the bundle sheath cell that is adjacent to the vascular bundle and are overlain by chloroplasts (Brown and Hattersley, 1989). This is thought to improve the efficiency with which CO2 released from mitochondrial glycine oxidation is reasimilated in the chloroplast. The mesophyll cells, which surround the bundle sheath, also contain functional chloroplasts, but do not have the ultrastructural organization of the bundle sheath cells. The mitochondria within the mesophyll cells have been found to lack GDC protein (Hylton et al., 1988; Moore et al., 1988; Rawsthorne et al., 1988, Devi et al., 1995). This means that glycine formed within the mesophyll cells has to cross to the bundle sheath in order to be metabolized, thus increasing the efficiency of CO2 reasimilation (Rawsthorne et al., 1988). It remains to be determined how this specialization arose. There would appear to be cell-specific factors regulating the expression of GDC in both C3 and C3–C4 plants. Although functional chloroplasts seem to be important in determining whether or not GDC is present in the cells of C3 leaves, this is not the case for C3–C4 intermediates where expression is somehow restricted to the bundle sheath, despite the presence of photosynthetically mature chloroplasts in the mesophyll cells. Although light is known to be an additional factor in regulating GDC expression (Oliver, 1994) it is unlikely to be the key factor in controlling the spatial distribution of GDC expression.

Recent work in this laboratory has shown that GDC is not the only mitochondrial protein to change in concentration during mesophyll cell development. Analysis
of over 30 different mitochondrial proteins, both mitochondrial- and nuclear-encoded, indicated three different patterns of development within barley primary leaves (Thompson et al., 1998). These were denoted Group A, B or C, according to their developmental profile. Of the proteins studied, all those involved in the electron transport chain or associated with its synthesis and assembly, including all mitochondrially-encoded proteins, showed a group A pattern of development. These proteins accumulated during the early stages of leaf development and reached maximum concentrations at the end of the zone of cell elongation (at 20.0 mm from the basal meristem). Included in this group were two respiratory enzymes, NAD-dependent malic enzyme and the E1β subunit of the pyruvate dehydrogenase complex (PDC). Group B proteins accumulated with increasing cell age, but at a slower rate than group A proteins, reaching a peak in concentration at the stage of leaf development where photosynthetic proteins, such as Rubisco, start to increase most rapidly. Group B proteins include the E1α subunit of PDC, NAD-dependent isocitrate dehydrogenase (IDH) and proteins involved in the transport of polyepptides into the mitochondrial matrix. Group C proteins, including the GDC protein subunits, increased throughout leaf development in a pattern similar to that of the photosynthetic proteins. Formate dehydrogenase and fumarase were also found to develop in this way. These distinct patterns of accumulation of proteins indicate that the composition of the mitochondria changes as the leaf cells develop and mature. In young leaf cells, lacking the capacity for photosynthesis, mitochondria will be essential for the provision of ATP. The early development of the mitochondrial electron transport chain and ATP synthase (Group A proteins) would be a fundamental requirement. With the onset of photosynthesis and photorespiration in mature leaf tissue, the co-ordinated increase in GDC subunits is essential for supporting the demands for glycine oxidation. As with seed germination, there is also evidence of the differential development of TCA cycle activity. The enzymes involved in the decarboxylatory portion of the TCA cycle (malic enzyme, PDC and IDH) reach a peak before those of the non-decarboxylatory portion (represented by fumarase). This, perhaps, marks a transition in the role of mitochondria from bioenergetic, in the early stages of autotrophic leaf development, to biosynthetic, as the leaf cells become photosynthetically competent (Tobin and Rogers, 1992).

A third, well-characterized developmental process that has provided a model for mitochondrial biogenesis studies is that of the Arum lily. It is of particular interest because, during flowering, the spadix tissue becomes thermogenic, due to the operation of the cyanide-resistant alternative oxidase (AOX). The biological function is to attract pollinators, as the rise in temperature stimulates the release of volatile compounds which attract flies (Vanlerberghen and McIntosh, 1997). Increased AOX activity coincides with an increase in AOX protein and mRNA in the spadix and a co-ordinated decrease in cytochrome oxidase expression. At its peak (D-day; the day of heat production and floral opening) the respiratory rate of the spadix is equivalent to that of the flight muscle of a humming bird and oxygen uptake is entirely cyanide resistant (Elston et al., 1989; Roads and McIntosh, 1992). Ultrastructural changes have been observed in the appendix of Sauromatum guttatum during anthesis. Skubatz et al. reported that the mitochondria began to divide, first in the epidermal cells (two days before D-day) and later in the subepidermal cells (on D-day) (Skubatz et al., 1993). The mitochondria also became more electron dense and this coincided with decreased cytochrome oxidase activity and increased rates of NADH oxidation. In Arum maculatum the activity of mitochondrial TCA cycle enzymes has been found to increase markedly during spadix development, indicating coarse control over TCA cycle activity (MacDougall and ap Rees, 1991).

Although AOX is present in virtually all plants, its physiological function in non-thermogenic species has until recently, remained unclear (for reviews see Lambers, 1982, 1985; Siedow and Moore, 1993; Moore et al., 1993; Vanlerberghen and McIntosh, 1994; Vanlerberghen et al., 1994; Wagner and Wagner, 1995). Developmental changes in AOX activity are not unique to thermogenic tissue. Increases in AOX mRNA have been detected during early pollen development in maize (Wen and Chase, 1999) and in common bean (Johns et al., 1993). Conflicting results have been reported during the ripening of climacteric fruits after harvest. In mango, AOX protein and mRNA increase (Kruz-Hernandez and Gomez-Lim, 1995), in contrast, protein and activity decrease in tomato (Almeida et al., 1999). AOX may be involved in ripening of tomato since ripening related genes of the non-climacteric capsicum when expressed in tomato, are induced during ripening but not when AOX inhibitors are applied (Kuntz et al., 1998). Developmental changes in AOX expression have also been found in vegetative tissue. Lennon et al. detected increases in AOX activity during pea leaf development due, partly, to conversion from the oxidized to the reduced (active) form of the AOX protein (Lennon et al., 1995). This increase in AOX activity coincided with a developmental increase in GDC activity and was considered to be related to a change from a bioenergetic to an increased biosynthetic function for the mitochondria (Lennon et al., 1995). Increased AOX activity has also been found during greening of the cotyledons of soybean seedlings (Obenland et al., 1970) and this coincides with increased AOX mRNA (McCabe et al., 1998). These developmental changes in AOX activity provide further evidence of differences in mitochondrial composition. They also illustrate the flexibility of mitochondrial activity. One proposed function for AOX in
non-thermogenic tissue is as an ‘energy overflow’ that allows the TCA cycle to continue to operate under conditions where the cytochrome oxidase pathway has become limiting, thus allowing replenishment of TCA cycle intermediates that have been directed into biosynthetic pathways (Mackenzie and McIntosh, 1999). This is consistent with the observed increase in AOX activity during photosynthetic development of pea (Lennon et al., 1995) and barley leaves (Thompson et al., 1998). A likely function for AOX in non-thermogenic tissue is that increasing oxygen is that it serves to protect the mitochondria from reactive oxygen species (ROS; e.g. H₂O₂ and superoxide) generated during electron transport. Maxwell et al. found that antisense suppression of AOX in tobacco cells resulted in a significantly higher level of ROS, while cells overexpressing AOX showed the opposite effect (Maxwell et al., 1999).

A similar function has been proposed for the uncoupling protein (PUMP) described above. Linoleic acid was found to decrease Δψ in isolated wheat mitochondria, thus indicating a fatty acid-translocating function for PUMP similar to that proposed for mammalian uncoupling proteins. This activity was stimulated by addition of ROS (hydrogen peroxide and superoxide) and linoleic acid was also found to reduce the mitochondrial production of ROS. This suggests that ROS can activate PUMP and that this functions to protect the cell against further ROS production (Pastore et al., 2000). Studies of AOX and PUMP during tomato fruit ripening indicate that these two processes operate at different developmental stages. Mitochondria isolated from green tomato fruit showed a higher state of coupling than those from red fruit, although PUMP was present in both. The difference was thought be due to the higher concentration of fatty acids in the red fruit, which resulted in a greater level of PUMP activity (Costa et al., 1999). The same group also found that linoleic acid inhibited AOX activity and reduced the ADP/O ratio in tomato fruit mitochondria. AOX was more sensitive to linoleic acid inhibition than was PUMP (which is activated by linoleic acid), indicating a differential ‘switch’ between the two energy dissipating pathways during fruit ripening. It was proposed that the two pathways operated sequentially, with PUMP occurring in the presence of high concentrations of fatty acids, such as in red tomato fruit, and AOX operating in unripe fruit (Sluse et al., 1998). If this is the case, there would have to be some purpose in changing from one pathway to another and, given that both processes apparently reduce ROS generation and also lower the ADP/O ratio, until some other function emerges, there is no obvious explanation for this transition.

In addition to these developmental studies, there are a number of independent reports of variations in mitochondrial structure and function in a range of different tissues. Dai et al. have found different populations of mitochondria in etiolated mung bean seedlings (Dai et al., 1998). Based on fractionation experiments, in which the mitochondria were separated by differential centrifugation and sucrose density gradients, four distinct populations of mitochondria were identified: regular sized orthodox mitochondria, poor respiring non-typical looking mitochondria (300–800 nm) and two populations of small ellipsoidal (70–300 nm) non-respiring slow sedimenting mitochondria. The DNA population in all the types of mitochondria identified was essentially the same. Although all populations possessed the mitochondrial marker enzyme cytochrome oxidase (Cox), its activity was consistently higher in the ‘orthodox’ mitochondria compared with the others. A range of both mitochondrially (Cox III, F₁ ATPase α) and nuclear encoded (porin, ATPase β) membrane enzyme mitochondrial proteins were detected in all four populations. In contrast the Elα subunit of PDC was found only in the orthodox and poor respiring mitochondria and not in the small ellipsoidal populations. The authors suggest that these small, slow-sedimenting mitochondria may represent ‘nascent mitochondria’ that undergo a gradual development in the growing seedlings. They also question whether these distinct mitochondrial populations indicate the presence of sub-classes of mitochondria, some performing routine physiological functions under normal growth conditions, and others whose primary function is to facilitate rapid bursts of growth at specific stages of development.

Comparison of photosynthetic and non-photosynthetic tissue of potatoes has identified marked differences in the protein composition of the mitochondria (Colas des Francs-Small et al., 1992). A total of 360 mitochondrial proteins were resolved by 2-D electrophoresis and both quantitative and qualitative differences were seen in photosynthetic as compared to non-photosynthetic tissue. Two hundred and eight of these, including 53 which remain unchanged quantitatively, were common to both tissue types. Etiolated tissues such as dark-grown shoots and roots, were more similar to each other in content and generally readily distinguishable from photosynthetic tissues. A number of proteins, including the four subunits of the GDC complex, were more abundant in photosynthetic tissues. Some proteins were more abundant in non-photosynthetic tissues, for example, a highly expressed 40 kDa soluble protein encoded in the nuclear genome resembling a NADP-dependent formate dehydrogenase, accounted for approximately 9% of total mitochondrial protein in non-photosynthetic tissues (Colas des Francs-Small et al., 1993). Until the proteins have been indentified it is unclear how these differences in protein composition relate to differences in mitochondrial function. Nevertheless, as an overview study, this provides further evidence of significant heterogeneity of mitochondria within different plant tissues.
Plastids: structure and function

Structure

Plastid is a general term applied to an important group of subcellular organelles found exclusively in plants. A variety of plastids occur in different plant organs during cellular development. All plastids are developmentally interrelated and interconvertible. Initially, all plastids are derived from small undifferentiated proplastids which are found in dividing meristematic cells. Depending on the particular cell type in which they are located and the stage of cellular development, these proplastids subsequently differentiate into a variety of specific plastid types. Although plastids may be thought of as a continuous spectrum of types, plastids may be either photosynthetic or non-photosynthetic. The photosynthetic chloroplast containing chlorophyll is found in all photosynthetic parts of a plant including leaves, stems, tendrils, and cotyledons. The chloroplast is also found in particular organs at specific stages of development e.g. unripe photosynthetic fruit (Blanke and Lenze, 1989).

A range of non-photosynthetic plastids have been characterized including the plastid precursor or eoplast (Thompson and Whatley, 1980), etioplasts and more specialized functionally mature plastids which fulfill a metabolic role within the cell, including chloroplasts, amyloplasts and elaioplasts. The nomenclature of plastids relates to their morphology, composition or localization and has been comprehensively reviewed elsewhere (Kirk and Tilney-Bassett, 1978; Thompson and Whatley, 1980).

Function

Plastids are involved in a range of metabolic processes including photosynthesis (Tobin, 1992), fatty acid synthesis, starch synthesis, nitrogen assimilation, and amino acid biosynthesis (Emes and Dennis, 1997; Neuhaus and Emes, 2000). These processes have been characterized in specific tissues and cells. Functionally, plastid heterogeneity is well described in photosynthetic tissue (Pyke, 1999). Immature leaf tissue is heterotrophic and needs to import carbon from mature leaf tissue or from seeds (Dale, 1985). With increasing maturity of chloroplasts there is an increased ability to photosynthesize and therefore to undergo predominantly autotrophic metabolism.

Clearly within a single leaf there are considerable changes in the rate of photosynthesis and the activity of photosynthetic enzymes. From the base of a cereal leaf to the tip is a gradient of leaf cells, from heterotrophic metabolism in non-photosynthetic cells to autotrophic fully photosynthetic cells at the leaf tip. The absence of detectable rates of photosynthesis at the leaf base reflects, at least in part, an inability of the proplastids to synthesize ATP during their early stages of development (Weber et al., 1984). Other limitations to the photosynthetic capacity indicate additional areas of heterogeneity within a tissue that has traditionally been considered to be homogeneous. Calvin cycle enzyme levels are relatively low in immature leaf cells. Rubisco protein is detectable in the basal cells of wheat (Dean and Leech, 1982) and barley (Viro and Kloppstech, 1980) leaves, but the amount per cell increases some 20-fold towards the tip (Dean and Leech, 1982). A number of components of the Calvin cycle have been shown to have correlations in their activity and photosynthetic rate including in wheat Rubisco (Besford et al., 1985; Ostareck and Lieckfeldt, 1989), phosphoglycerate kinase (PGA kinase) and fructose 16 bisphosphatase (FBPase) (Suzuki et al., 1987).

It is possible to illustrate with selected examples the potential heterogeneity that exists within non-photosynthetic plastids. Already some plastid heterogeneity in relation to nitrogen metabolism has been discussed elsewhere (Tobin and Yamaya, 2001). Here it is the intention to illustrate different degrees of heterogeneity of proteins and genes between plastids, both at the level of different plastid types and also where potential differences within what has traditionally been described as the same plastid population may occur.

Heterogeneity of plastid form and function

Plastid phosphate translocators

Communication between plastids and the surrounding cytosol occurs via the plastid envelope membrane. More specifically, a variety of inner membrane transporters mediate the exchange of metabolites (Flügge, 1999). It is now clear that metabolites are not transported by a single transport system, but by a set of different members of the phosphate translocator family with partially overlapping substrate specificities. This allows the efficient uptake of individual phosphorylated substrates even in the presence of high concentrations of other phosphorylated metabolites. This multiplicity of transporters relates to specific plastid types and differences are seen even within the same plastid population, reflecting the flexibility of plastid metabolism. Carbon fixed during the day can be exported from the chloroplasts into the cytosol in the form of triose phosphates via the triose phosphate/phosphate translocator (TPT; Flügge, 1999). The need for phosphoenolpyruvate, as an immediate precursor for the synthesis of secondary products via the shikimic acid pathway or as a precursor for fatty acid or aromatic amino acid biosynthesis, necessitates a plastidic phosphoenolpyruvate/phosphate translocator (PPT; Fischer et al., 1997). Non-photosynthetic plastids must import carbon in the form of hexose phosphates via the glucose 6-phosphate/phosphate translocator (GPT; Kammerer et al., 1998). TPT, PPT and GPT proteins share a high degree of identity with each other (75-95% identical), but only
about 35% similarity between members of the different transporter families (Kammerer et al., 1998). The different physiological functions of the phosphate translocator families are linked to differential expression patterns. The TPT gene is predominantly present in tissues that perform photosynthetic carbon metabolism. The release of newly synthesized carbohydrates occurs by the export of triose phosphates catalysed by the TPT and strictly coupled to the uptake of Pi (Flügge, 1999). The PPT gene is ubiquitously expressed, though its transcripts are more abundant in non-green tissue (Fischer et al., 1997).

The GPT gene is barely detectable in photosynthetic tissues, but abundant in heterotrophic tissue, particularly those which utilize Glc6P for starch synthesis such as potato tubers, maize kernels and pea roots (Kammerer et al., 1998).

In addition to the obvious differences in localization between autotrophic and heterotrophic plastids there is additional information available to support the idea that some transporter activity is restricted to specialized cells. For instance, in photosynthetic tissue it has been suggested that PPT may be present in only a subtype of the mesophyll chloroplasts (Flügge, 1999). Under normal growth conditions chloroplasts do not possess the capacity for Glc6P transport. However, GPT has been identified in the envelope of guard cell chloroplasts (Overlach et al., 1993). The guard cell chloroplast possesses a functional light harvesting apparatus, but only limited CO₂ fixation capacity and a lack of FBPase (Heidrich et al., 1985). Therefore for a turnover of starch during the opening and closing of stomata, then like non-green plastids, hexose phosphates must be imported from the cytosol. As such, hexose phosphate transport is not a function of the capacity for energy provision within the organelle, but reflects the carbohydrate metabolism. This is further confirmed when examining the capacity for GPT in chloroplasts of spinach leaves. If detached leaves were fed glucose, inducing a rapid switch from autotrophy to heterotrophy, then they were seen to exhibit GPT activity (Quick et al., 1995). This raises the possibility that the type of translocator found in any tissue may be flexible and specifically reflect the metabolic demands placed upon that tissue. A switch to a functional GPT in chloroplasts may be in response to the metabolism of the detached leaf in glucose becoming a sink rather than a source organ. This hypothesis is reinforced by the observation that the amount of mRNA encoding TPT protein is strongly decreased after feeding sucrose to tobacco seedlings (Knight and Gray, 1994). Whether there is a change in the balance of transporters in all the chloroplasts or only a selected population is at present unknown. With CAM induction in Mesembryanthemum there is a marked increase in the enzyme activities of starch degradation and glycolysis, and the export of hexose phosphates across the chloroplast envelope of Mesembryanthemum crystallinum is induced (Kore-eda and Kanai, 1997; Neuhaus and Schulte, 1996).

Starch synthesis in storage tissues from monocotyledonous plants

In developing endosperm of some cereals the enzyme ADP-glucose pyrophosphorylase (AGPase) is found in both the cytosol and the amyloplasts (Denyer et al., 1996; Emes et al., 1999; Thorbjørnsen et al., 1996). AGPase is a heterotetramer comprised of large and small subunits. Although the amyloplast form of the large subunit is present throughout wheat endosperm development, the cytosolic form appears only after 10 d post anthesis (MM Burrell, unpublished information). This not only implies developmental regulation of the two spatially distinct forms, but also has important implications in terms of starch synthesis within the amyloplasts. When AGPase is localized exclusively in the amyloplasts, hexose phosphates via the GPT and ATP via an ATP/ADP transporter must be transported into the amyloplast for its activity. In contrast ADPglucose formed via a cytosolic AGPase activity later in development must be imported into the plastids for starch syntheses via an ADP-glucose/adenylate antiporter, bypassing the need for Glc6P and ATP import for starch synthesis. An ADPGlc transporter expressed in development at approximately the same time as the cytosolic subunits for AGPase has been purified in wheat endosperm (IJ Tetlow, CG Bowsher, MJ Emes, unpublished results). The implications in terms of the presence of different transporters in the same or different amyloplasts during development needs to be determined.

Metabolic interactions

There is clear heterogeneity between non-photosynthetic plastids from different sources, at present it is unclear whether there is any variation between those from the same source and within the same population. Storage plastids carry out several different anabolic and catabolic processes simultaneously (Neuhaus and Emes, 2000), by necessity the reactions influence each other and are integrated and as such require a complex mixture of metabolites. Studies have tended to consider individual metabolic processes and not linked the complexity of the situation in vivo, where multiple substrates may be being used by more than one process simultaneously.

For instance using wrinkled-seeded pea embryos the r locus mutation leads to a decreased starch content and a significantly increased level of storage and structural lipids, suggesting these processes are inversely linked (Bettley and Smith, 1990). Isolated cauliflower bud amyloplasts are physiologically competent
for acetate-dependent fatty acid synthesis and Glucose 6-phosphate (Glc6P)-dependent starch synthesis (Neuhaus et al., 1993). Since both metabolic pathways require ATP to drive the conversion of the specific precursors to form the starch and fatty acid end-products, then the interaction between these pathways can be examined. At external ATP levels which saturate Glc6P-dependent starch synthesis, acetate driven fatty acid synthesis is only at approximately 30% of its maximal rate. Where starch synthesis is activated by the allosteric regulator of AGPase, PGA, fatty acid synthesis decreases by approximately 55% at limiting (0.5 mM) and 32% at saturating (4 mM) ATP concentrations (Mühlmann et al., 1994).

The pathway of carbohydrate oxidation within the amyloplast may also represent a drain on the flux of carbon to starch. In wheat endosperm amyloplasts the oxidative pentose phosphate pathway (OPPP) is stimulated when the demand for reducing power is made by glutamate synthase during amino acid production. The diversion of hexose phosphates (Glc1P) to sustain carbohydrate oxidation via the OPPP leads to a 75% reduction in starch synthesis (Tetlow et al., 1994). A similar competition of metabolic processes between nitrite reduction and glutamate synthesis has been characterized in pea root plastids and again illustrates the integrated metabolism within non-photosynthetic plastids (Bowsher et al., 1996). Such competition for substrates between metabolic processes is probably indicative that these processes are occurring within the same plastid.

In contrast a different interaction is seen during the early mid-stage of oilseed rape embryo development, where again fatty acid and starch synthesis can occur at the same time (Kang and Rawsthorne, 1996). Embryo plastids although photosynthetic are not thought to be capable of net CO₂ fixation in vivo due to the pod wall shielding the tissue from photosynthetically active radiation (Eastmond et al., 1996). Purified oilseed rape embryo plastids supplied with Glc6P, pyruvate and ATP show a 3-fold increase in fatty acid synthesis with no affect on starch synthesis suggesting that these pathways do not compete for ATP. Furthermore, in vitro studies of plastids show that when Glc6P and pyruvate are supplied either alone or together then the total flux to fatty acid synthesis is greater when supplied together than alone. This could be indicative that individual plastids within the population used for the experiment differ in their capacity for Glc6P and pyruvate use for fatty acid biosynthesis. That is, some plastids have a substantially higher capacity for Glc6P utilization relative to pyruvate and others use pyruvate better than Glc6P. If either substrate is supplied individually then only a proportion of the plastid population is capable of utilizing the supplied metabolites. Therefore a significant amount is produced when both substrates are supplied together and all the plastids can make fatty acids.

**Plastid heterogeneity**

Plastid heterogeneity in terms of specific plastid types in particular tissues or in cells at different stages of development is well characterized and has already been discussed. For the purpose of this review plastid heterogeneity can also be used to describe heterogeneity within a particular class of plastids. For instance Larsson et al. used a dextran-polyethylene glycol 2-phase system to isolate two morphologically distinct intact chloroplast populations from a broken chloroplast population (Larsson et al., 1971). Class I and class II intact chloroplasts were rich in stroma and surrounded by an envelope, but class II intact chloroplasts had an additional extra-cytoplasmic-like membrane. The space between the chloroplast envelope and the extra membrane varied, but it could be sufficiently large to contain cytoplasmic material including mitochondria, peroxisomes and ribosome-like particles. At the time it was suggested that class II chloroplasts were found in distinct cells, from class I chloroplasts. More specifically, the enriched class II chloroplast fraction was isolated when mid-vein and stalk tissue was used to isolate the chloroplasts.

Studies using root tissue have also suggested there is heterogeneity of plastids within the root. Newcomb first reported the presence of cup-shaped plastids, sections of these revealed regions of the cytosol enclosed by the plastid (Newcomb, 1967). Läuchli et al. identified various root plastid shapes where the inner membrane was invaginated or only slightly developed (Läuchli et al., 1974). Root plastids from the cortical parenchyma and vascular stele cells were examined using light and electron microscopy (Peat and Tobin, 1996; Tobin and Yamaya, 2001). From these studies it appeared that a variety of root plastid forms were identifiable. Although the shape was often rounded and irregular or dumb-bell in form, four distinct classes of root plastids were identified: (i) those with no discernible internal organization; (ii) root plastids with no distinct globular membranous regions; (iii) single internal flat membrane; (iv) those with well-defined starch grains (usually epidermal plastids).

**Regulation of organelle heterogeneity**

As the examples described above show, there is a diversity of organelle form and function within plant tissue. An understanding of the regulation of this process of differentiation may give a greater insight into the significance of this heterogeneity and its importance in regulating metabolism. Both the mitochondria and plastids have their own genome, but the majority of their proteins are encoded within the nuclear genome. This evolutionary step, from autonomy to nuclear integration, means that organelle biogenesis can be co-ordinated with cell division and development. The co-ordination
of nuclear and organellar gene expression is, thus, a fundamental control of organelle form and function. Although there is much to be discovered about these control mechanisms, it is becoming increasingly evident that transcriptional regulation is important in regulating organelle differentiation.

**Mitochondria**

To date RNA polymerases (RNAP) genes have not been identified in the mitochondrial genome of photosynthetic eukaryotes. Recent evidence supports the presence of one or more nuclear encoded mitochondrial RNAPs (Hedtke et al., 1997). In order to initiate transcription efficiently, mitochondrial RNAPs also require transcriptional factors to recognize and bind to their promoters. It has been suggested that there may be a role for additional tissue-specific proteins able to regulate or modulate RNAP activity according to the requirements of different tissues and specifically associated with a high number of mitochondrial promoters in plants. Although mitochondrial transcription factors (mtTF) have been identified in a range of organisms, to date no plant factors with homology to animal or yeast mtTF have been identified. There is now a need to investigate whether they can function as specific factors and as activating factors in plants. In the young leaves of *Triticum aestivum* and depending on the developmental stage of particular cells the abundance of several mitochondrial gene transcripts has been shown to be inversely regulated when compared to those in the chloroplast (Topping and Leaver, 1990). Similarly, the transcript levels of mitochondrial genes are higher in white, photosynthetically inactive leaves as opposed to green leaves of *albostrians* mutants (Börner and Hess, 1993). The molecular mechanisms underlying the observed differences in transcript levels are not clear and the contribution of post-transcriptional processing and mRNA stability has yet to be understood.

Further evidence of a close association between chloroplast development and mitochondrial protein composition has come from work on mutants as well as experiments using chemical treatments to disrupt normal plastid development. The *albostrians* mutant of barley, for example, has ribosome-deficient plastids and this prevents synthesis of a range of chloroplast proteins. Affected leaf tissue is white and the plastids lack thylakoids and chlorophyll. Expression of mitochondrially-encoded genes was found to be affected, with enhanced expression of genes encoding cytochrome oxidase subunits (*coxII, coxIII*) and ATPase (*atpA, atp6, atp9*). It was shown, by crossing the mutants with wild-type barley, that the increase in transcript levels was due to the lack of chloroplast development and was not a result of the nuclear *albostrians* allele. This was also supported by the effect of bleaching by treatment with norflurazon, which led to impaired chloroplast development and also enhanced the level of mitochondrial transcripts. These studies indicate that plastid development can affect mitochondrial gene expression (Hedtke et al., 1999).

**Plastids**

Plastid genes are transcribed by two different types of RNAPs, one of the eubacterial type of plastid encoded RNAP (PEP) and one nuclear encoded polymerase (NEP) (Hess and Börner, 1999). It has been suggested that PEP promoters use promoters of a type that are not recognized by NEP and vice versa (Hajdukiewicz et al., 1997; Hübschmann and Börner, 1998). NEP transcribes mainly housekeeping genes whilst PEP, although transcribing some housekeeping genes, mainly transcribes photosynthetic genes. NEP is the major RNAP in plastid nongreen tissues and is essential for normal plastid function. However, PEP lacking plastids are unable to construct the photosynthetic apparatus (Hess and Börner, 1999).

Higher plants consist of numerous plastids which fulfill a range of different functions. Since many of these plastids are photosynthetically inactive then there is no need to transcribe genes that encode products exclusive for photosynthesis. It would therefore appear that the primary function of NEP is to transcribe genes at possibly a low rate to maintain the plastids as a compartment for its other biosynthetic functions. Generally, these processes are driven by nuclear encoded enzymes in higher plants. At the same time, a nuclear encoded RNAP not only maintains the metabolic activity of these non-photosynthetic plastids but poises proplastids ready to develop into chloroplasts. PEP lacking plants have plastids and plastid DNA at nearly normal levels (Hess et al., 1993). Once chloroplast or etioplast development starts, NEP activity increases and specific NEP promoters are activated in order to enhance transcription of rpo genes and other housekeeping genes. This is supported by the observation that the highest accumulation of rpo transcript is at the base of the cereal leaf, where the proplastids and plastids are in the early stage of development to photosynthetically active chloroplasts (Baumgarten et al., 1993; Inada et al., 1996). Later as the chloroplasts differentiate, PEP dominates the transcript providing the necessary capacity for the expression of photosynthetic genes (Mullet, 1993). In fully developed chloroplasts NEP remains active to transcribe genes without PEP promoters and also genes which at the same time are transcribed by PEP (Allison et al., 1996; Hajdukiewicz et al., 1997; Hübschmann and Börner, 1998; Iratni et al., 1997). To date there is no evidence characterizing this regulation further, but one could envisage that the differential activation of NEP and PEP and their
promoters, respectively, should be regulated. Transcriptional regulation in this manner could regulate the role of plastids within a given population, so that specific plastids are concerned with particular metabolic roles, for instance, amino acid biosynthesis or fatty acid metabolism.

Conclusion

The biochemical studies of purified organelles from different species and tissues provides important information. However, this information must be considered as of only limited value since it most likely represents not a homogeneous population, but a heterogeneous mixture of organelles derived from different cell types. Clearly experimental evidence is now becoming increasingly available to suggest that there are distinct differences between organelles located in different tissues and cells. The availability of techniques to examine these differences routinely (and highlighted in this special issue) means that it should now be possible to study the function and interactions of individual organelles in the metabolic context of a particular cell environment. The potential of such approaches has yet to be fully realized, but once applied should contribute in a significant way to advance the understanding of the complexity of mitochondrial and plastid heterogeneity.

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