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The Proteins of *Tulipa* and Their Relation to Morphogenesis[1]

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INTRODUCTION

The relations between genetics and biochemistry on the one hand and growth, development, and morphogenesis on the other are salient problems of modern biology. The changes in the complement of soluble proteins that occur concomitantly with development in the tulip bulb permit these relationships to be described. The technique of acrylamide gel electrophoresis furnishes the means to show the range of proteins that occur and the ways in which they change; the special morphology of the tulip bulb, in which the complete transition from a quiescent, vegetative shoot to a fully formed flower all occurs within the bulb, presents for study a unique range of accessible and controllable morphogenetic events.

A widely accepted doctrine is that the synthesis of specific proteins is gene controlled. Therefore, whenever protein changes occur during development both the morphogenesis and the biochemistry should reflect the extent to which gene action is regulated.

Furthermore, normal development poses the obvious problem that cells which were all derived equationally from a zygote must, in different organs, make very different use of their identical genetic information. Mounting evidence that mature but freed cells of angiosperms can give rise to whole organisms (Steward *et al.*, 1966, and references

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there cited) testifies that their genetic integrity is conservatively preserved throughout development. Therefore, certain stimuli must activate, or repress, the regions of chromosomes which secrete mRNA's into the cytoplasm and cause the formation of specific proteins. Thus the orderly events of development may now be seen in terms of the sequential formation of the appropriate proteins at the time and place appropriate for the organism's development. The changes in the protein complement along the axis of pea roots and in seedlings, as first investigated by Brown and Robinson (1955) and later by Steward *et al.* (1965), are compatible with this view; the information that now accrues from the study of the tulip relates to a much wider range of morphogenetic events.

In insects the hormone that regulates morphogenetic development of the larvae, ecdysone, causes visible evidence of activity in the form of chromosomal puffs at characteristic places on the chromosomes, and this response can be correlated with the animal's development. There is no adequate parallel in higher plants for the polytene chromosomes of insects. Nevertheless it is a plausible hypothesis that morphogenetic stimuli in plants (like those that stimulate the onset of flowering) may operate in a similar way. Therefore, any evidence that links morphogenetic behavior to protein metabolism may be a useful step in linking development to gene action.

HISTONES

It is not only the structural proteins of cells that may conceivably be organ specific, for enzyme actions may be mediated by different proteins (isozymes) that are characteristic of different organs. This concept has been developed for animal systems by Markert (1963a, 1965).

In a previous study (Zacharius *et al.*, 1957), changes in the composition of the alcohol soluble, nonprotein, nitrogen compounds (amino acids and amides) were noted as vegetative tulip bulbs developed flowers. The environmental stimuli that evoke the formation of flowers in otherwise mature, but vegetative, tulip bulbs are well known (Hartsema, 1961); in general prior treatment at 68°F[2] is required to initiate flowers, whereas subsequent storage at 48°F permits them to develop normally, although different temperature regimes can cause abnormal flowers. It is also convenient that light is not involved in this

[2] The Fahrenheit scale is used throughout because of its common use in these greenhouse practices.

transition and that much of the growth of the tulip flower occurs within the bulb, which is, moreover, self-sufficient for the nutrients that support growth. Thus, the cultivated tulip (*Tulipa gesneriana*) is a favorable plant on which to investigate the protein composition of different organs and to trace the changes that accompany floral induction and flower development.

The bulb scales of the mature bulb constitute a series of similar organs in which the protein content in relation to development may be investigated; this may be done while the bulb is at rest, or again as its stored reserves are being mobilized for the growth of the flower or in the formation of next year's tulip bulb. But an opportunity to relate protein complements to morphology occurs when the proteins found in the vegetative organs (foliage leaves or bulb scales) of the main axis are compared with those found in their modified counterparts in the flower, i.e. the tepals (sepals and petals), stamens and pistils. More dramatically, the transition from the vegetative to the flowering condition presents the opportunity to relate proteins to morphogenesis by determining the composition of shoot apices before and after the floral induction that occurs under sharply defined storage conditions. It is the results of such a study that are to be presented.

MATERIALS AND METHODS

Growth of the Bulbs and Sampling Procedures

A preliminary investigation of the soluble proteins of *Tulipa* was performed using rooted bulbs (*Tulipa gesneriana* cultivar Greenland) obtained from local sources. Flowers were already initiated within these bulbs when they were received; fifty of them were washed and dissected into root tips (1 mm long), whole roots, bulb scales, lateral buds, tepals, anthers, and pistils.

In a more comprehensive study fifty bulbs of *T. gesneriana* cv. Golden Harvest, which were initiated when obtained, were dissected as described above, but their tepals were subdivided into sepals and petals. Comparable bulbs were grown under greenhouse conditions in natural light at 55°F. After 40 days in the greenhouse the bulbs were in full flower, and fifty plants were again sampled and dissected into their various parts. Although fifty bulbs yielded adequate amounts of protein from most of their parts, it was necessary to use a still larger sample when the shoot apex was to be analyzed. The apex was isolated

without any adhering basal plate or bulb scale tissue. Microscopical examination of the shoot apices as dissected from the bulbs five months after planting, proved that they were still vegetative. The bulbs were then transferred to 68°F conditions and on alternate days thereafter bulbs were examined and, if floral initiation had commenced, the stage of development was noted. The events of floral initiation were somewhat synchronized since approximately 60% of the bulbs were simultaneously at a particular stage of development, while the remainder deviated only slightly. Table 1 shows the status of the shoot apices at the different times of sampling. The various stages are shown in Fig. 1 a–d. The plants at the different stages were also dissected into roots and individual bulb scales.

TABLE 1

STATUS OF BULBS OF *Tulipa gesneriana* CV. GOLDEN HARVEST PRIOR TO AND DURING FLORAL INITIATION AND DEVELOPMENT

5 months after planting	Foliage from current year dead. Growing points in the new bulbs vegetative but immediately prior to initiation. Bulbs transferred to 68°F
2 weeks at 68°F	One or both whorls of perianth visible, i.e., the stages designated P_1 and P_2[a]
3 weeks at 68°F	Anthers partially or completely differentiated, i.e., the stages designated A_1 and A_2[a]
4 weeks at 68°F	Gynoecium differentiated, i.e., the stage designated G[a]

[a] The convention of Beyer (1942) to designate the various stages of floral initiation has been adopted here.

Bulbs from a further supply of *T. gesneriana* cv. Golden Harvest were grown to flower under greenhouse conditions at 55°F and sampled for protein analysis of their various parts. Other bulbs were left until the foliage had matured, when they were transferred to 68°F. By periodic sampling, dissection, and microscopical examination of the apices, the time course of floral initiation was again followed. From such material one hundred vegetative apices were removed immediately prior to their initiation and another hundred apices at the first sign of floral initiation, i.e., when the primordia of the first whorl of the perianth became visible. The elapsed time between these samplings was 6 days.

The intention was to dissect the various flower part primordia from the axis; however sufficient material for this purpose could be obtained

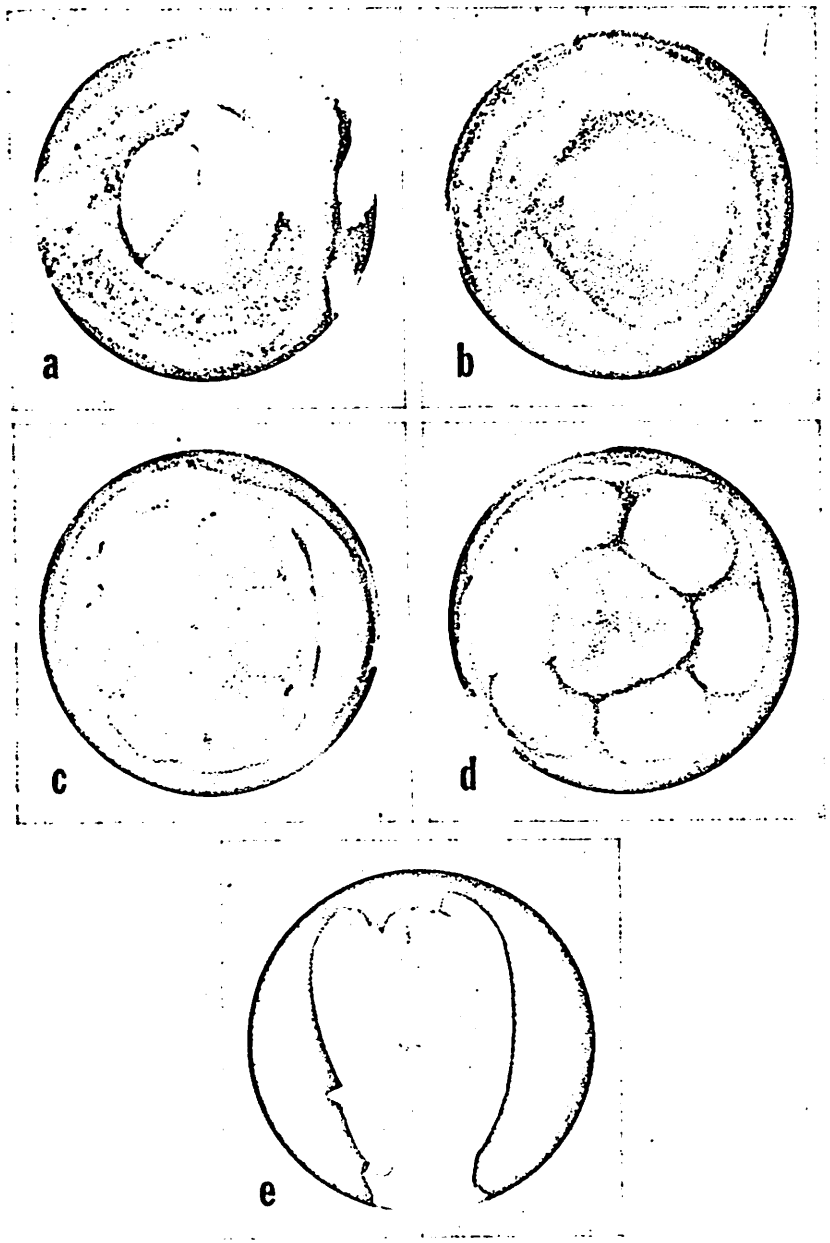

FIG. 1. Flower formation in *Tulipa gesneriana*. Flowers were initiated at 68°F and subsequently matured at 48°F. (a) A normal vegetative shoot apex in surface view showing the apical dome and the latest leaf primordium; 0 days. (b) A similar apex after 14 days of initiation (stage P_2), both whorls of perianth present. (c) An apex after 21 days of initiation (stage A_2), both whorls of anthers present. (d) An apex after 28 days of initiation (stage G), three carpels present. (e) An immature flower dissected from a bulb after 4 weeks of initiation and 2 weeks of development. Magnification: a–d, ×21; e, ×7.

Figures a–d are from an earlier study by Cathey (1955) working with one of us (F.C.S.).

only by pooling material available from five cultivars of *T. gesneriana* which were obtained from the Cornell test gardens. Equal numbers of each of the five closely related cultivars comprised the pooled population. Samples were so obtained that they were accurately matched. All these bulbs were brought by appropriate means to the point of floral induction. One hundred bulbs were sampled while their apices were still vegetative; a similar batch, 6 days later, when the first whorl of the perianth appeared; and again, 5 weeks later, when the perianth was approximately 4 mm long, a further two hundred bulbs were sampled. The relevant stages are shown in Fig. 1. From the last sample, the apices of eighty-five bulbs were used intact while the remainder were dissected further into the various immature flower parts.

Proteins were extracted from each of the samples so obtained, and acrylamide gel electrophoresis was performed in the manner to be described.

Preparation of Extracts and Electrophoretic Techniques

The various samples obtained from bulbs of different ages were extracted immediately after harvesting. Comparable amounts of tissue were used for each extraction so that the aliquots applied to each gel represented approximately equal amounts of tissue and contained of the order of 200 μg of soluble protein. Electrophoretic separation was in 7.5% acrylamide gels at pH 8.3 as described by Steward and Barber (1964) and Steward *et al.* (1965). The separated proteins were routinely stained using 0.7% (w/v) amido black in 7% (v/v) acetic acid, and the resultant gels show how the different proteins were distributed. It is appreciated that this standard procedure reveals a given range of proteins which is useful for comparative purposes; by means of other procedures, proteins with different properties could have been revealed (cf. Barber *et al.*, 1967). From time to time more specific tests were used to characterize certain of the bands revealed by amido black. These procedures, which for the most part are modifications of stand-

ard histochemical techniques, were those developed for the localization of alkaline phosphatase activity (Beckman and Johnson, 1964), malic acid dehydrogenase activity (Goldberg, 1963), and esterase activity (Markert and Hunter, 1959) using α-naphthylbutyrate as the substrate.

After staining, the gels were washed repeatedly with 7% (v/v) acetic acid, and the results are presented in the ways previously used by Steward *et al.* (1965).

RESULTS

Figure 2 shows comparable acrylamide gel electrophoretic separations of the soluble proteins extracted from the different parts of bulbs of *T. gesneriana* cv. Greenland. It is obvious that the complement of soluble proteins present at any one time varies from organ to organ; this is so despite the fact that bulb scales, foliage leaves, tepals, and even anthers and pistils may all be regarded as modified leaves composed of cells with the same genetic information. Each organ has therefore a characteristic protein complement which may be specific for the organ in question at that particular stage in its development. Certain organs (bulb scales and axillary buds) are more similar than others (roots and tepals). Moreover, in some parts of the plant body the soluble proteins were concentrated in very few bands (e.g., root tips).

Figure 3 presents the data from a given gel in three ways. Interpretative diagrams of the gels (Fig. 3c), which summarize all the information from visual inspection (Fig. 3b) and densitometric examination (Fig. 3a) of the gels, may be assembled to form a "map" (Fig. 4). The map shows the ways in which the complement of soluble proteins varies from organ to organ. Again (cf. Fig. 2) each organ appears to have a protein pattern which characterizes that organ at the particular stage of development at which it was sampled. The material used to obtain the results of Fig. 2 was of a different cultivar at a slightly different stage in development from that used to obtain the results of Fig. 4. Nevertheless there were certain general similarities: e.g., in both cases (Figs. 2 and 4) there were few protein bands in the pistils, and they were present in small amounts; by contrast there were many slow-moving bands in the foliage leaves and in the perianth. While the sepals and petals of *Tulipa* are indistinguishable and are collectively referred to as tepals, they can be distinguished as outer (sepals) and inner (petals) whorls and, as such, they also have different protein

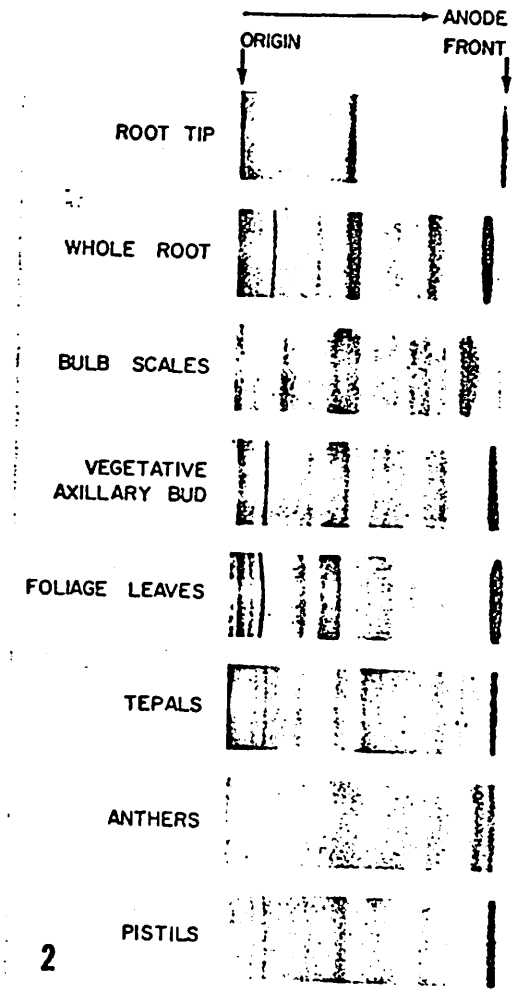

FIG. 2. The electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Greenland; showing the complement of proteins present concurrently in the different organs. The leaves and floral parts were immature and were dissected from within the bulb.

complements (Fig. 4) which collectively represent the protein pattern of the tepals (Fig. 2). Similarly, the various whorls of bulb scales (which together comprise a developmental sequence) have different and characteristic protein patterns (Fig. 4), but collectively they

represent the protein pattern of the bulb scales as a whole (cf. Fig. 2).

Figure 5 shows the progressive changes in the soluble proteins as the vegetative shoot apices were induced and as floral parts were initiated. Also shown are the protein patterns of the mature flower. It can be seen that the vegetative apex just prior to initiation contained a minimum amount and number of soluble proteins, but as induction occurred and floral parts were initiated, the numbers of different bands and their intensity increased. Some of these newly synthesized proteins appeared to be the same as those which were to be seen later in the mature floral organs. In other words there were indications that the

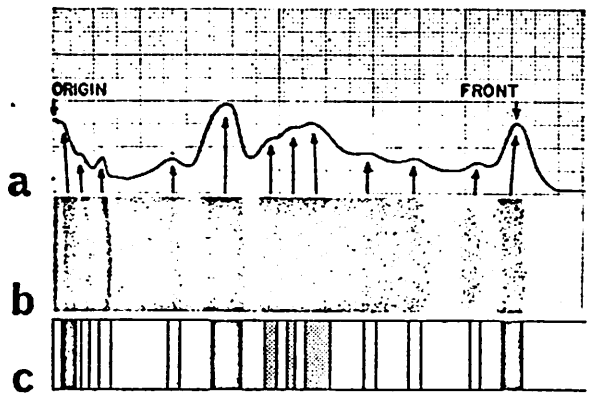

FIG. 3. Methods of presenting the results of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa*, using an extract from foliage leaves dissected from within the bulb (cf. Fig. 2). (a) A densitometric trace from the gel shown in the photograph at (b). (c) Interpretative diagram consolidating the information at (a) and (b) and from visual examination of the gel.

appearance, on the apex, of the different flower part primordia was accompanied by the appearance of some of the proteins characteristic of mature flowers. For example, of the two bands (R_f 57 and 59) which were present only in the mature anthers, only one (R_f 59) was represented in the vegetative apex. This band was intensified as initiation occurred, and in the fully initiated apex (stage G) both bands (R_f 57 and 59) were revealed in larger amounts. Other similar examples will become evident on inspection of Fig. 5.

Figures 2, 4, and 5 clearly show that floral induction and development are associated with recognizable changes in the complement of soluble proteins; tests were therefore made to see whether the changes correlate

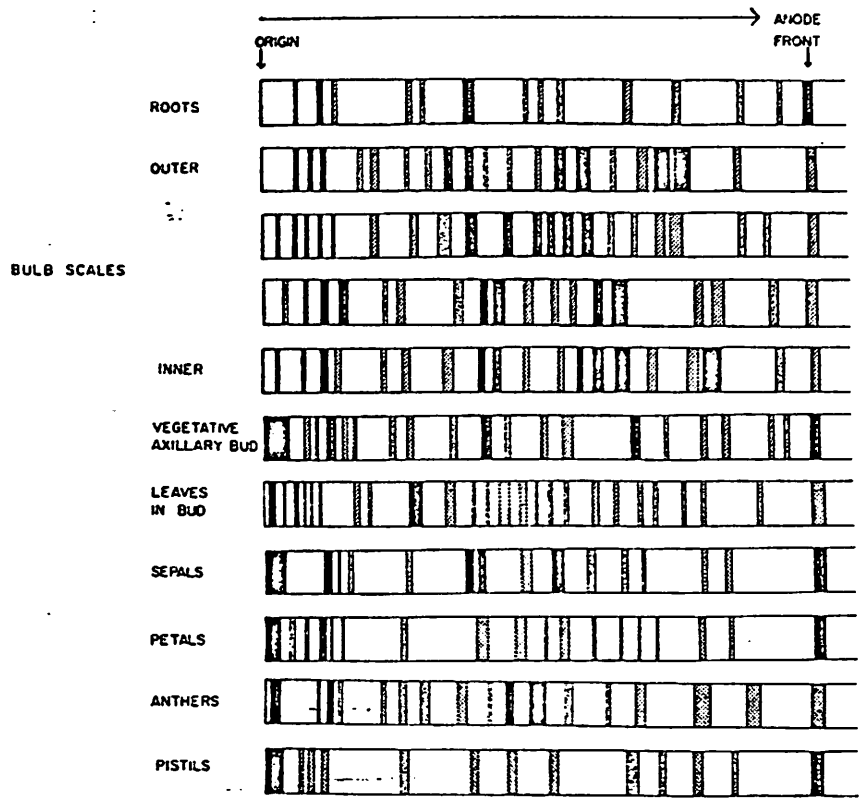

FIG. 4. Diagrammatic interpretations of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing the complement of proteins present concurrently in the different organs. The leaves and floral parts were immature and were dissected from within the bulb.

in proteins arose after the floral induction stimulus but well before the floral organs had matured. The results are shown at Fig. 6. To obtain these results larger numbers of apices than used hitherto were necessary. Apices were dissected from vegetative bulbs (cf. Fig. 1a) and 6 days later from bulbs subjected to the floral stimulus. By this time the florally induced apices had not yet reached the P_2 stage of Fig. 1b, for this would have required approximately a further 7 days at 68°F. In response to these treatments new protein bands appeared, especially in the region closest to the origin, in the otherwise vegetative apices (cf. Fig. 6a and b with c and d). The amounts of protein used for gels

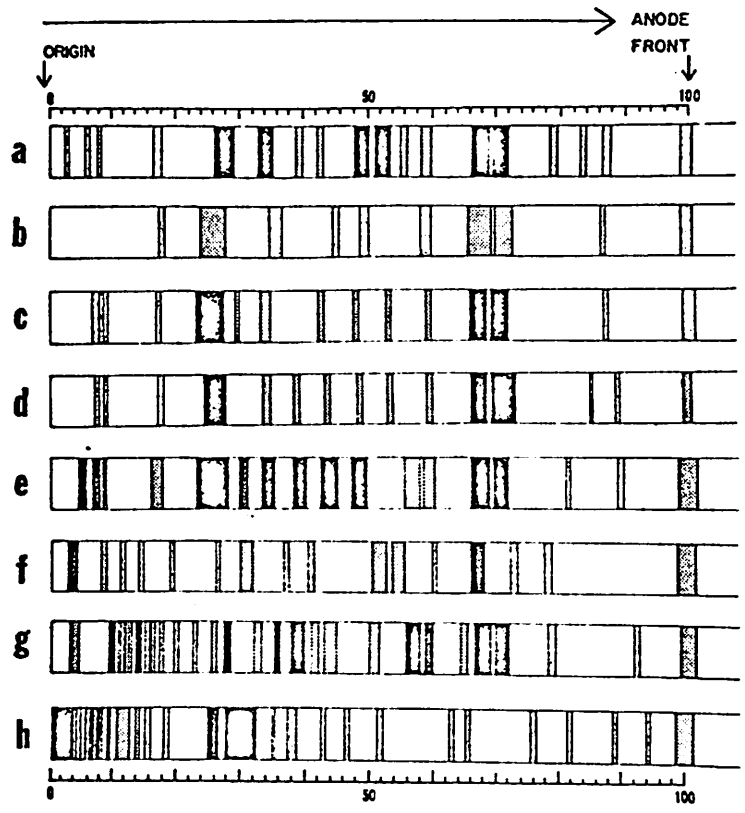

FIG. 5. Diagrammatic interpretations of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing the complement of proteins present in the shoot apices at various stages of floral initiation and in the mature flower parts. (a) Vegetative shoot apices from bulbs in full flower. (b) Vegetative shoot apices immediately prior to floral initiation. (c) Shoot apices with one or both whorls of perianth distinguishable (stages P_1 and P_2). (d) Shoot apices with one or both whorls of anthers distinguishable (stages A_1 and A_2). (e) Shoot apices with three carpel primordia distinguishable (stage G). (f) Mature tepals. (g) Mature anthers. (h) Mature pistils.

of Fig. 6, and the photography, deliberately emphasized the slow-moving bands which appeared after floral induction even though these devices rendered the others somewhat more diffuse. The major changes observed in response to floral induction occurred in bands that could easily be detected visually, appeared on the tracings, and were to be

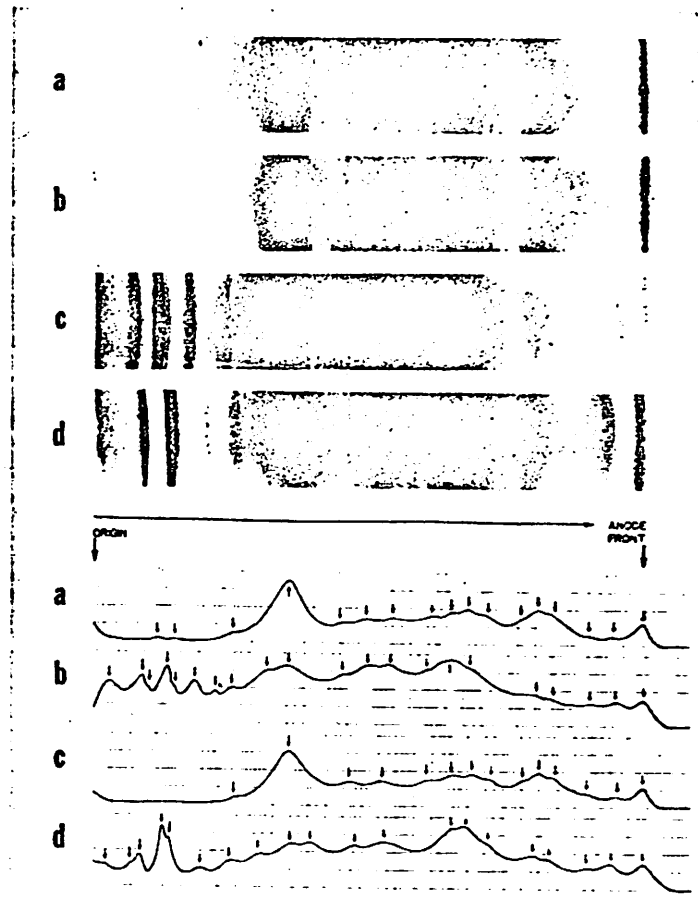

FIG. 6 (top). The electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa;* showing the complement of proteins present in the shoot apex before and after floral induction at 68°F. (a) Vegetative shoot apices from accurately matched samples of five cultivars of *T. gesneriana.* (b) Vegetative shoot apices of *T. gesneriana* cv. Golden Harvest. (c) Induced shoot apices from accurately matched samples of five cultivars of *T. gesneriana* after 6 days of induction. (d) Induced shoot apices of *T. gesneriana* cv. Golden Harvest after 6 days of induction.

FIG. 7 (bottom). Densitometric traces of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa;* showing the complement of proteins present in the shoot apex before and after floral induction at 68°F. (a) Vegetative shoot apices from accurately matched samples of five cultivars of *T. gesneriana.* (b) Induced shoot apices from accurately matched samples of five cultivars of *T. gesneriana* after 6 days of induction. (c) Vegetative shoot apices of *T. gesneriana cv.* Golden Harvest. (d) Induced shoot apices of *T. gesneriana* cv. Golden Harvest. (d) Induced shoot apices of *T. gesneriana* cv. Golden Harvest after 6 days of induction.

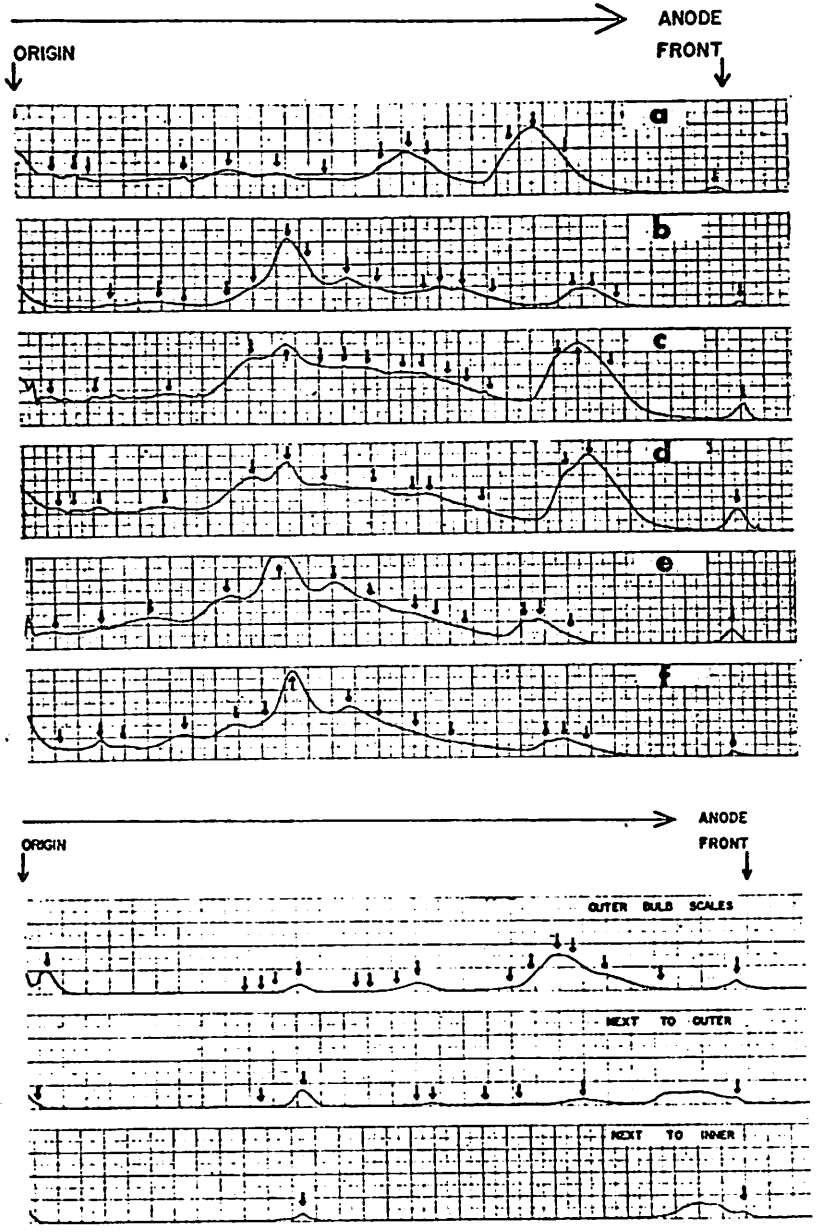

FIG. 8 (top). Densitometric traces of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing the complement of proteins present in the inner bulb scale at various stages of development of the shoot apex. (a) Shoot apex of bulb was vegetative. (b) Shoot apex of bulb was vegetative but immediately prior to induction. (c) Shoot apex of bulb had initiated two whorls of perianth. (d) Shoot apex of bulb had initiated two whorls of anthers. (e) Shoot apex of bulb had initiated three carpel primordia. (f) Two weeks after complete initiation of the shoot apex.

FIG. 9 (bottom). Densitometric traces of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing the complement of proteins present concurrently in the bulb scales of plants in full flower. The absorption peak immediately behind the front in the lower two traces was due to a brown pigment, not to proteinaceous material. At this stage in development the innermost bulb scale was completely depleted of extractable protein.

seen in the photographs. Even so some other proteins previously present seemed to change in their relative amounts. These observations could be made on the single cultivar (Fig. 6b and d) or on the pooled cultivars (Fig. 6a and c). In fact the responses observed in tulip apices due to floral induction were remarkably free from varietal differences, so that the data of Fig. 6 also demonstrate the reproducibility of the methods.

The protein changes due to floral induction which can be seen in the apices (Fig. 6) can also be observed in the densitometric traces of Fig. 7. In Fig. 7, a and b represent the vegetative and florally induced apices, respectively, from the pooled cultivars, and they compare with a and c of Fig. 6. Similarly c and d of Fig. 7 represent vegetative and florally induced apices, respectively, from the single cultivar and they compare with b and d of Fig. 6. (Wherever an arrow appears on the tracing it could be supported by a visible band, even though faint, on the corresponding gel.) The appearance of the conspicuous slow-moving bands in the gels from the florally induced apices (i.e., Fig. 6c and d) is clear on their corresponding tracings (Fig. 7b and d). The conspicuous bands in the vegetative apices (Fig. 6a and b) changed during floral induction; this can also be seen in the amplitude of the corresponding peaks of the tracings in Fig. 7. Thus the results of Figs. 6 and 7 show that detectable changes occur in the soluble proteins of the apex in response to the floral induction stimulus and that these are evident as soon as the first signs of visible morphogenetic change.

While the effect of the floral stimulus is apparent in the soluble proteins of the shoot apex, Fig. 8 shows that it is also to be seen in the soluble proteins of the inner bulb scales. During floral initiation certain proteins decreased or disappeared, possibly because of their

mobilization and translocation to the developing bud, while others progressively accumulated. The changes in the form of the densitometric traces of Fig. 8 a–f show this. Figure 9 shows that during flowering there was a preferential removal of soluble protein from the inner bulb scales as compared with the outer ones, for at this stage the innermost bulb scale was totally depleted of soluble protein while the outer whorls contained increasing, but nevertheless small amounts of soluble proteins. Figure 9 also shows that certain proteins were more selectively removed from the bulb scales during flowering than others.

Figures 10–13 show that the conclusions which have been drawn from the changing complement of soluble proteins as revealed by amido black may be corroborated by more specific tests for enzyme proteins. Tests for alkaline phosphatases, malic acid dehydrogenases, and esterases showed bands that were distinct from those revealed by amido black (Fig. 10). It is now known that amido black does not stain

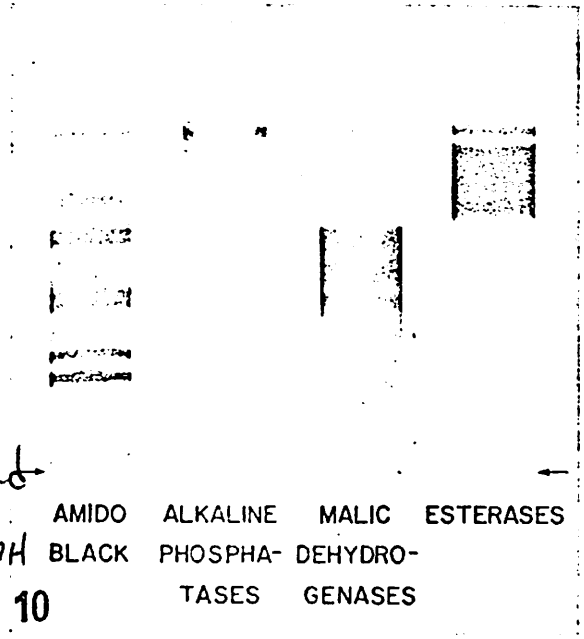

FIG. 10. The electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing replicate gels, of an extract of the vegetative axillary bud, which were stained in the various ways indicated. The origins of the gels (shown thus →) are toward the bottom of the page and the anode toward the top.

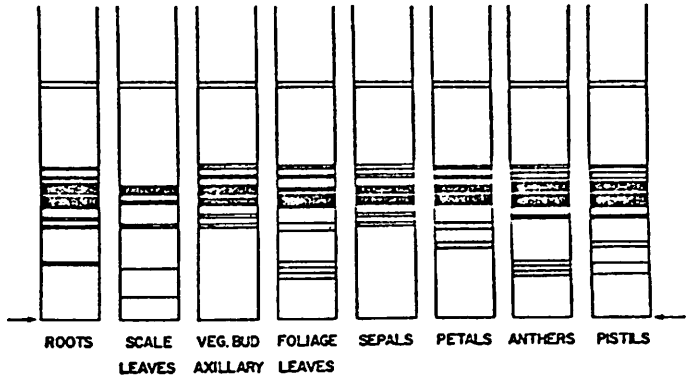

FIG. 11. Diagrammatic interpretations of the electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing the malic acid dehydrogenases which were present concurrently in the different organs. The leaves and floral parts were immature and were dissected from within the bulb. The origins of the gels (shown thus →) are toward the bottom of the page and the anode toward the top.

all the proteins that are present in a given gel, and even the appearance of a single band may often represent several proteins.

Enzyme tests therefore provided the further opportunity to study the effects of morphogenesis in terms of proteins with more rigorously defined properties. Both the malic acid dehydrogenases (Fig. 11) and esterases (Fig. 12) varied with the organs, vegetative and floral, from which they were extracted. The tissue or organ specificity was most apparent for the malic acid dehydrogenases. In a given organ there were 4 to 7 isozymes of malic acid dehydrogenase, a total of 9 isozymes being detectable. While the tissue specificity as shown by the esterases was not so striking, nevertheless all the mature floral organs contained one esterase (the band nearest the origin) which was absent from, or undetectable in, the vegetative parts.

Figure 13 shows that when vegetative (Fig. 13a) and reproductive (Fig. 13b) apices were dissected from bulbs of the cultivar Golden Harvest certain esterases disappeared in response to the floral stimulus. More interesting, however, was the appearance of the esterase band which had been present in the mature floral organs but which, prior to this, was absent from the vegetative parts. Using vegetative apices (Fig. 13c) from the accurately matched samples of five different cultivars and their corresponding reproductive apices (Fig. 13d), 6 weeks after floral induction a similar result was noted. The floral response

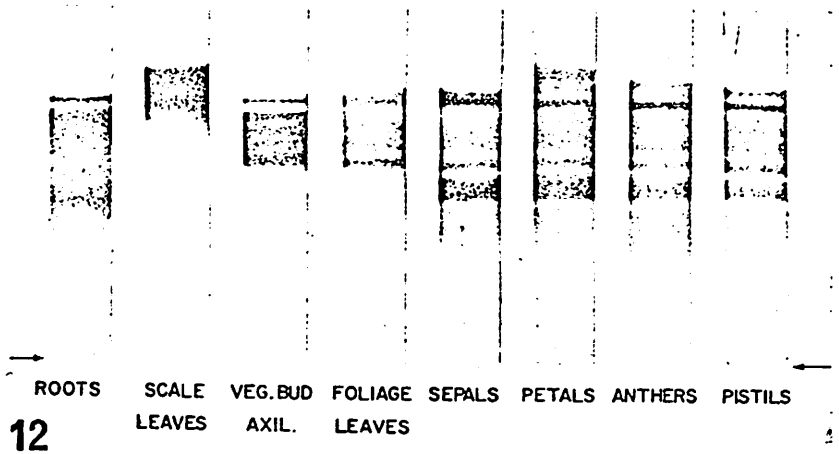

FIG. 12. The electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa gesneriana* cv. Golden Harvest; showing the esterases which were present concurrently in the different organs. The leaves and floral parts were immature and were dissected from within the bulb. The origins of the gels (shown thus →) are toward the bottom of the page and the anode toward the top.

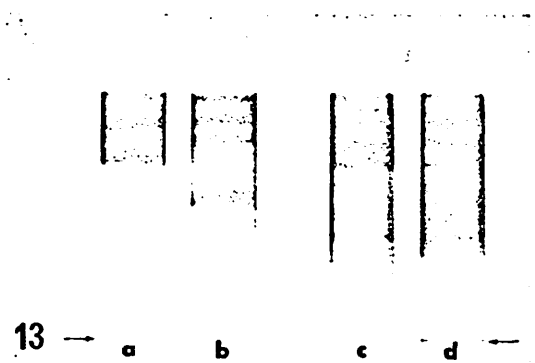

FIG. 13. The electrophoretic separation on acrylamide gels of the soluble proteins of *Tulipa;* showing the esterases of the shoot apex before and after floral induction at 68°F. The origins of the gels (shown thus →) are toward the bottom of the page and the anode toward the top. (a) Vegetative shoot apices of *T. gesneriana* cv. Golden Harvest. (b) Induced shoot apices of *T. gesneriana* cv. Golden Harvest after 6 days of induction. (c) Vegetative shoot apices from accurately matched samples of five cultivars of *T. gesneriana.* (d) Induced shoot apices from accurately matched samples of five cultivars of *T. gesneriana* 6 weeks after floral induction.

was, therefore, linked to concomitant changes in the esterases of the shoot apex; an esterase band which in the mature bulbs was only present in the floral organs (cf. Fig. 12) appeared promptly in the florally induced growing points.

An attempt was made to distinguish between the esterases of the primordia of the different floral organs when these were dissected from the growing point. The most distinctive esterase band which was characteristic of the flower parts (cf. Fig. 12) was present in all the primordia, and indications that other esterases were specific for different flower part primordia were suggestive but not conclusive.

DISCUSSION

This study of the soluble proteins of the tulip has proved rewarding because the range of proteins observed at any one time is so different in the different organs and also because the proteins are so subject to change throughout the annual cycle of growth (cf. Figs. 4–6). The tulip bulb and flower have therefore, presented an opportunity to relate the behavior of proteins to growth and development.

The proteins present in mature but dissimilar organs (roots, bulb scales, floral parts, etc.) are understandably different (cf. Figs. 2, 4, 5, 11, and 12). Nevertheless the dilemma here is to distinguish between differences in protein patterns which are the consequence of morphogenesis and those by which it may be causally determined. It is for this reason that the changes observed in the shoot apices before and after floral induction are so significant. The evidence is that the protein complement responded to the conditions that induce flowering before the floral organs had developed (cf. Figs. 5, 6, 7, and 13). Others have claimed that there is more total protein in florally induced apices than in vegetative ones (e.g., Gifford, 1963) or have stressed that such differences in proteins are quantitative, not qualitative (Marushige and Marushige, 1962; Nitsan, 1962).

However, the consequences of the conditions that induce flowering are not confined to the apical growing regions for they extend to the proteins present in the already mature bulb scales. The fact that tulip bulbs must reach a critical size before they will flower implies that the bulb scales participate in the floral response. In *Iris*, Rodrigues Pereira (1962, 1964) implicated both primordial leaves and bulb scales in the flowering response. Therefore the bulb scales of tulip may play a more intimate role in the development of flowers than the mere supply of

QUESTION Good

nitrogenous nutrients for they also respond to the floral stimulus by changes in the nature and the amount of the soluble proteins which they contain (cf. Fig. 8).

Organ-specific proteins, or those that arise in response to such morphogenetic stimuli as those that induce flowering, require specific mRNA's to activate their formation. Evidence for these has not yet been obtained in the systems in question, but it should be sought. An effective explanation along these lines requires that the genes involved in flowering should be repressed in vegetative bulbs and derepressed in those that are florally induced. A current idea, derived from Stedman and Stedman (1950), is that the histone chromosomal "coat" causes genes to be repressed (cf. Huang and Bonner, 1962). Arginine-rich histones have been shown to inhibit nuclear RNA synthesis in some systems (Allfrey *et al.*, 1963). It is therefore interesting that the large amount of alcohol-insoluble, bound arginine present in the resting tulip bulb is so subject to change as the floral organs develop (Zacharius *et al.*, 1957). The evidence along the above lines is still somewhat tenuous for angiosperms. Gifford (1964) invoked histochemical procedures to show that there is generally more RNA in florally induced apices of *Chenopodium, Pharbitis,* and *Xanthium* than in their vegetative counterparts. Cherry and Van Huystee (1965) claim that photoperiodically induced *Xanthium* buds incorporate more labeled uridine into mRNA than buds of noninduced plants. Many investigators now involve RNA's in all hormone-like responses of plants (cf. Van Overbeek, 1966).

The first event of chemically induced growth of cultured carrot explants is a great increase in RNA and ribosomes per cell, though this is rendered effective in growth and protein synthesis only by the later effect of growth factors that cause cell division (Steward *et al.*, 1964).

It is fair to say that while differences in protein composition that accompany differentiation and morphogenesis are clear, their causal relationship to specific mRNA's, by which their formation may be regulated, is much less firmly established.

While the general protein stain amido black suffices to reveal a wide range of reproducible electrophoretically separable protein bands, it certainly cannot differentiate all the soluble proteins that are present. Thus the proteins that are specifically related to morphogenesis may not have been sensitively revealed by the amido black procedure. The use of stains that are specific for proteins capable of acting as enzymes

serves to restrict attention to proteins with a given function and therefore, renders any organ-specific differences more precise. The successful application of tests to detect alkaline phosphatases, malic acid dehydrogenases, and esterases among the soluble proteins of the tulip bulb has therefore led to more clear-cut differences between the content of these enzymes (e.g., malic acid dehydrogenases and esterases) in mature vegetative and floral organs and also, as a response to floral induction, in the growing points.

Enzymatically catalyzed reactions which are virtually universal in living cells (e.g., malic acid dehydrogenase) may nevertheless be mediated by organ-specific proteins when the enzyme in question exists in the form of several isozymes. Markert (1963b) recognized five electrophoretically separable isozymes of lactic acid dehydrogenase in developing bovine tissue and attributed these five isozymes to tetramers built from two distinct monomers, the production of which is controlled by two nonallelic genes. In this way lactic acid dehydrogenase activity can be mediated by distinctive organ-specific proteins. Markert and Sladen (1966) were able to detect nine lactic dehydrogenase isozymes in penguin heart tissue and concluded that the four additional bands were due to "unexplained deviations" in one or more of the five major isozymes. Goldberg (1966) recognized nine such isozymes in a hybrid fish (speckled × lake trout) but to explain these, like Blanco and Zinkham (1963), he suggested a third subunit controlled by a third nonallelic gene.

Following Markert (1963b) there have been several reports of five malic acid dehydrogenase isozymes in animals and plants (Staples and Stahmann, 1963; Moore and Villee, 1963), and recently Patton *et al.* (1967) have reported the presence of seven malic acid dehydrogenase isozymes in unfertilized sea urchin eggs. In *Tulipa,* however, the number seems to be nine (cf. Fig. 11), which requires an explanation similar to that of Markert and Sladen (1966), or, that there are three distinct subunits responsive to three nonallelic genes. Alternatively the plant may be a hybrid and is heterozygous at one of the gene loci responsible for one of the subunits. While it might be desirable to dissociate the dehydrogenase polymers, this would be a separate study. Nevertheless, and whatever the causes, the presence of multiple bands of malic acid dehydrogenase in *Tulipa* could permit these enzyme functions to be exercised in organ-specific ways, and it is especially significant that the stimulus for flower formation produces, in the

growing points, the appropriate enzyme proteins long before the mature floral organs have developed. This seems to follow clearly from the comparison of Fig. 6a and b with e and d. In mice, Markert and Hunter (1959) also correlate changes that occur during differentiation of organs with concomitant increases in the number and amounts of esterases which are present.

Thus the observations made on the soluble proteins of tulip support the general view that differentiation and morphogenesis are accompanied by the formation of organ-specific proteins and enzymes. Since even mature, diploid plant cells seem to be essentially totipotent (Steward *et al.*, 1966) some form of regulatory control of protein synthesis must intervene, and this control is usually sought at the level of the genes and is believed to be mediated by mRNA's. Even so the presumably small molecules, which transmit the effects of environment from the region of "perception" to the genes at the site of action, still need to be found. Such precise chemical links between the temperature stimulus that controls morphogenesis and the proteins which form in the resultant organs of the tulip are still to be sought. While certain protein changes in the growing point anticipate the formation of floral organs, and may be presumed to be causally related to the development that ensues, it is still to be shown that they owe their formation to specific mRNA's which originate when genes are derepressed by the floral stimulus. Further work is needed to establish this otherwise convenient working hypothesis.

SUMMARY

The complement of soluble proteins in the different organs of the tulip plant at different stages in the growth cycle has been investigated. This has been accomplished by the application of acrylamide gel electrophoresis. A general protein stain (amido black) and others which are specific for given enzyme proteins have been used. By these methods it has been demonstrated that the organs present at any one time in the bulb exhibit organ specific protein complements; these change with time, and this is especially shown by the contrast between vegetative and floral organs. In particular the mature vegetative and floral organs showed marked contrasts with respect to the enzyme proteins for which tests were made, i.e., isozymes of malic acid dehydrogenase and esterase. Thus the question arises whether the floral stimulus, sensitively controlled by temperature, elicited the formation of the appropriate proteins before, or after, the organs were formed. Hence studies were made on shoot apices before and after floral induction. The evidence shows that marked changes in soluble proteins occur so soon after floral induction as to justify the view that they precede the morphogenetic responses. Thus, by taking advantage of the special features of the tulip bulb, of the extent to which its morphogenetic responses may be controlled, and of the fact that the entire development of the flower occurs within the bulb and, by applying to this system the technique of acrylamide gel electrophoresis, it has been possible to demonstrate intimate relationships between the proteins as synthesized and the organs as they develop. The significance of these results is discussed in terms of current views of protein synthesis and of morphogenesis.

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