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Regulation of gibberellin formation by the fungus *Gibberella fujikuroi*

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Abstract. Gibberellins are a classic example of the production of plant growth regulators by microorganisms. They are important biotechnological products and are increasingly used in agriculture and horticulture. The economic importance of these plant hormones has led to an extensive study of the regulation of gibberellin biosynthesis. There have been reports of light, growth rate, inoculum size and carbon and ammonium sources acting as regulators of gibberellic acid biosynthesis. Besides light stimulation, nitrogen repression is a well-known regulatory principle of secondary metabolite formation. In *Gibberella fujikuroi* ammonium interferes with the production of gibberellic acid whereas phosphate does not influence the biosynthesis. It was found that the negative effect of ammonium ions is due to both the inhibition of activity and the repression of *de novo* synthesis of specific gibberellin-producing enzymes. Besides nitrogen control, the biosynthesis of gibberellins is suppressed by glucose. This glucose effect can be overcome by the addition of mevalonic acid. Therefore, the key enzyme of the isoprenoid pathway, the HMG-CoA reductase, seems to be the target of C-catabolite repression. A detailed knowledge of the regulation of gibberellin biosynthesis is important for fermentation processes. The biological function of gibberellin formation for the producing fungus is discussed.

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The gibberellins (GA) are a group of plant growth hormones that were first isolated from the fungus *Gibberella fujikuroi* (imperfect stage, *Fusarium moniliforme*), a widespread phytopathogenic fungus often found on corn, rice, barley and other crops in different geographical regions throughout the world. Besides gibberellins, *G. fujikuroi* produces a number of interesting biologically active secondary metabolites, including pigments, phytotoxins and mycotoxins (Brückner et al 1989).

Mevalonic acid is the structural unit of many secondary metabolites, including the gibberellins, the carotenoids and the trichothecene toxins. Mevalonic acid is first converted via the isopentenyl, geranyl and farnesyl pyrophosphates and then further metabolized to the different secondary metabolites. Gibberellins

have many functions in plants. They stimulate stem elongation, induce flowering and overcome seed dormancy. Because of the economic importance of the gibberellins as plant hormones, industrial researchers have established the conditions in which gibberellins can be produced and have looked for *G. fujikuroi* strains that synthesize increased amounts of these hormones.

Knowledge of the regulation of gibberellin biosynthesis is very important for the optimization of culture conditions. Up to now, little has been known about the regulation of gibberellins. There have been reports that light, ammonium ions, carbon source and growth rate all act as regulators of gibberellin biosynthesis in *G. fujikuroi*.

Zweig & De Vay (1959) first reported that 'cultures which were kept in darkness usually produced less gibberellins than cultures which were kept in the light'. Mertz & Henson (1967) and Mertz (1970) found a light-stimulated increase in acetate incorporation which could be prevented by AMO-1618, an inhibitor of GA biosynthesis which acts at the step of *ent*-kaurene synthetase. Johnson & Coolbaugh (1990) showed that the difference between levels of GA production in light- and dark-grown daughter cultures was greater if the two-day-old stock cultures had been maintained in darkness. Light-grown and older inocula (5–6 days) appeared to lose their capacity for light stimulation of GA synthesis. Furthermore, these authors found that the kaurenoic acid oxidation appears to be light stimulated. Rates of oxidation to products having GA-like properties were increased by up to 60% in enzyme preparations from cultures grown in light.

Interestingly, blue light has been found to stimulate isoprenoid biosynthesis in several of the Ascomycete fungi. Carotenogenesis in *G. fujikuroi, Blakeslea trispora* and *Neurospora crassa* is markedly increased, more than 300-fold, by blue light irradiation. Johnson & Coolbaugh (1990) suggested that light-stimulated GA biosynthesis also involves blue light reception.

Avalos and co-workers (1988) studied the biosynthesis of carotenoids and gibberellins in wild and mutant strains of *Gibberella fujikuroi*. They found that wild-type carotenoid formation is stimulated 2.9-fold, whereas kaurene synthesis is increased 438-fold. In contrast, enzymic activity of the regulatory mutant is even higher in dark-grown mycelia. Therefore, light affects the total amount of terpenoids formed by *G. fujikuroi*.

The negative control of gibberellin A_3 (GA_3; gibberellic acid) biosynthesis by rapidly utilized carbon sources was well established early on by Borrow et al (1961, 1964). An initial concentration of glucose in the culture of more than 3% results in a low yield of GA_3, and at this glucose level there is a lag phase that is otherwise absent (Vass & Jefferys 1979). These effects were overcome by feeding glucose in such a way that the concentration at any time was less than 4%. Mevalonic acid seems to be an initiator in the production of GA_3. Feeding of the prime precursor under conditions of catabolic repression overcomes the lag period. GA_3 production starts a short time after the addition of mevalonate. Therefore, the key enzyme in the

isoprenoid pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), is likely to be one, or the only, target of catabolic regulation.

The inhibitory effect of ammonium ions on gibberellin biosynthesis by *G. fujikuroi* has been known for a considerable time and was first reported by Borrow et al (1964).

We have studied the nitrogen regulation of GA synthesis in some detail (Brückner & Blechschmidt 1991). Figure 1 shows the influence of $(NH_4)_2SO_4$ concentration on the yield of GA_3 in defined and complex media. In defined medium, optimal production of GA_3 occurred at 22.5 mM $(NH_4)_2SO_4$. In a complex medium containing corn steep liquor, the conditions for *G. fujikuroi*

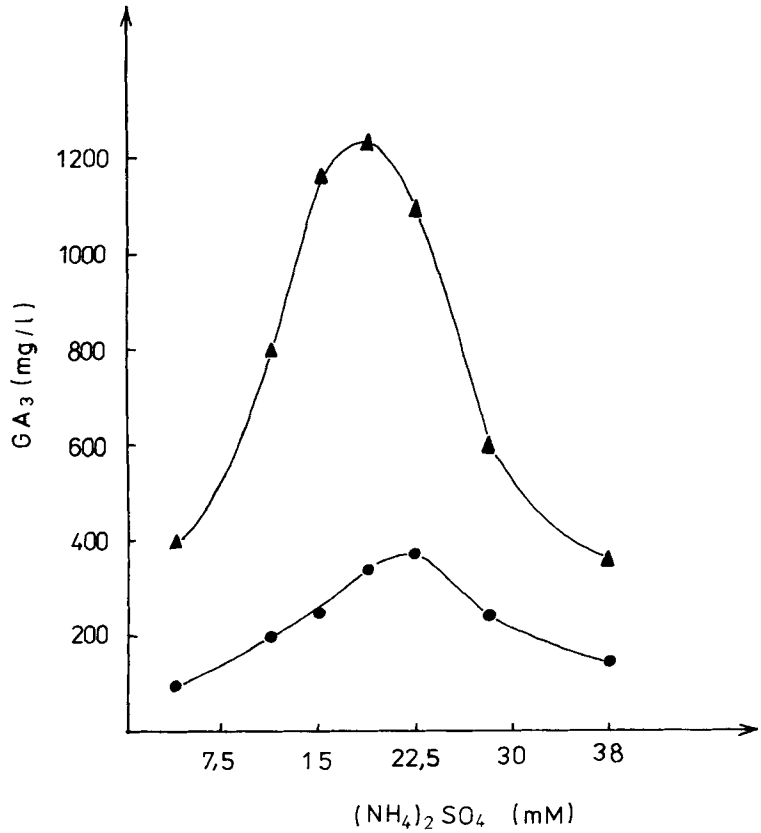

FIG. 1. The effect of $(NH_4)_2SO_4$ concentration on the maximum gibberellic acid (GA_3) titre in defined (filled circles) and complex (filled triangles) media.

growth and for the production of GA_3 are much better. The maximum GA_3 titre was measured in medium containing 19 mM $(NH_4)_2SO_4$. With a further increase in nitrogen concentration, the formation of GA_3 was decreased drastically. Figure 2 shows the effect of ammonium excess (38 mM $(NH_4)_2SO_4$) on fungal growth and gibberellin formation in comparison to optimal (19 mM $(NH_4)_2SO_4$) production conditions. The synthesis of GA_3 was reduced three-fold in the medium at high ammonium concentration, whereas growth was not greatly affected. Under these conditions, a significant amount of ammonium still remained in the culture fluid after 168 hours. Nevertheless, the formation of GA_3 began at the same time as it did in the medium containing the lower concentration of $(NH_4)_2SO_4$. These results are in contrast to the findings of other authors (Borrow et al 1961, 1964, Bu'Lock et al 1974, Vass & Jefferys 1979), who reported that GA_3 production began only when, or soon after, the nitrogen source in the medium had been exhausted.

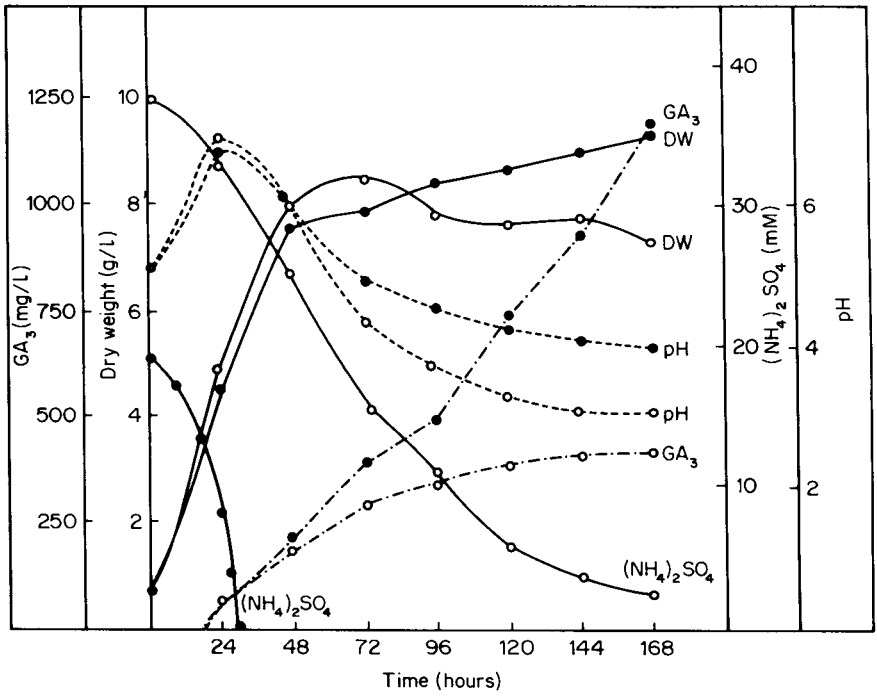

FIG. 2. Effect of optimal (19 mM) (filled circles) and high (38 mM) (open circles) $(NH_4)_2SO_4$ concentrations on the growth of *G. fujikuroi* (DW, dry weight), gibberellic acid production (GA_3) (—·—·—) and pH (----) in a complex medium. The concentration of $(NH_4)_2SO_4$ over time is shown.

To investigate further the effect of ammonium ions on biosynthetic enzyme activity, we added a high level of $(NH_4)_2SO_4$ (38 mM) to complex medium cultures at different times after GA_3 production had started. The medium already contained 19 mM $(NH_4)_2SO_4$. Figure 3 shows that the biosynthesis of gibberellic acid (GA_3) was nearly completely inhibited a few hours after the addition of ammonium sulphate. In another experiment, the onset of gibberellin biosynthesis in complex medium containing a high or low concentration of $(NH_4)_2SO_4$ was studied by adding cycloheximide (100 μg/ml) to batch cultures

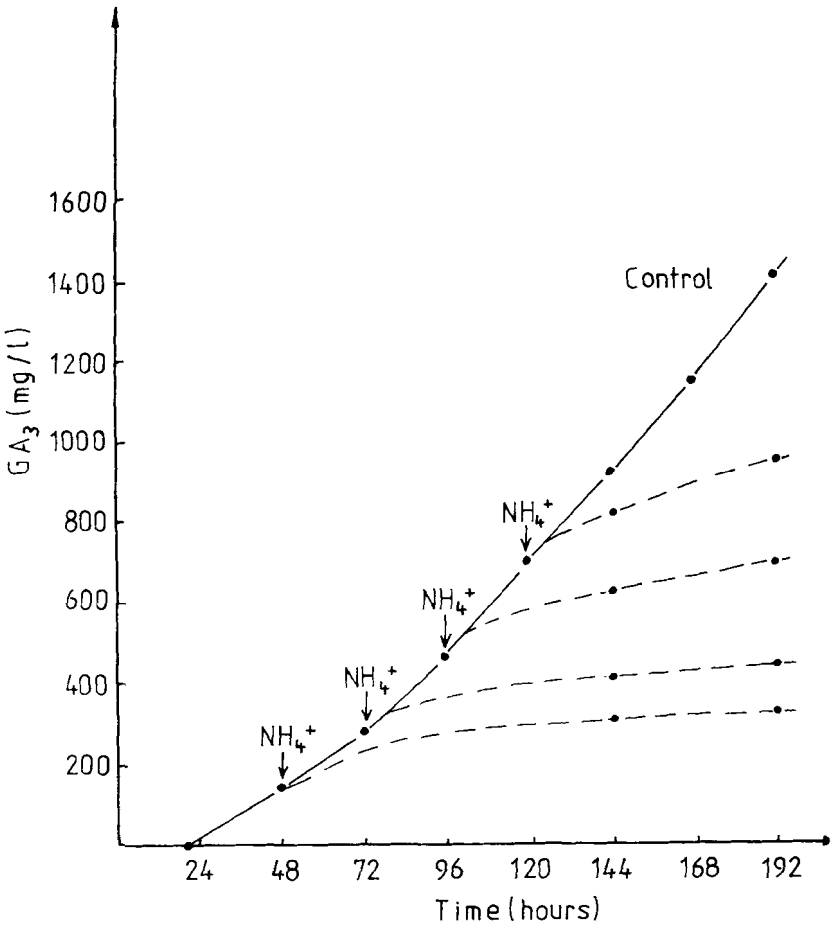

FIG. 3. Influence of the addition of 38 mM ammonium sulphate (arrows) on the production of gibberellic acid in a complex medium containing 19 mM $(NH_2)_2SO_4$ as the major nitrogen source.

of various ages. Addition of this protein synthesis inhibitor at 17 hours or 20 hours prevented a further increase in mycelial weight, and no gibberellins were formed. Addition of cycloheximide at 24, 30 or 41 hours allowed a short phase of gibberellin biosynthesis. Therefore, the enzymes of gibberellin pathway are not constitutive but were produced in the first 20 hours of cultivation.

To determine the specific gibberellin productivity during fermentation under different conditions, we prepared resting cell suspensions with washed mycelia previously grown on one of two concentrations (19 mM or 38 mM) of $(NH_4)_2SO_4$. The resting cell activity (mg GA_3 per mg mycelium per hour) of mycelia which had been grown at both concentrations of $(NH_4)_2SO_4$ increased during growth, reached a maximum after 72 hours, and fell during the stationary phase (Fig. 4). The activity of the gibberellin biosynthetic enzyme complex of cells previously grown in 38 mM $(NH_4)_2SO_4$ was lower than that in cells from an ammonium-limited medium (19 mM $(NH_4)_2SO_4$). This indicated that one or more enzymes in the gibberellin biosynthetic pathway were repressed—that is, their own production was reduced in the presence of ammonium ion. Moreover, the addition of $(NH_4)_2SO_4$ to the resting cell system led to a strong decrease in resting cell GA synthetic activity. This indicates that the negative effect of NH_4^+ on gibberellin synthesis is due to repression *and* to the direct inhibition of one or more enzymes in the gibberellin biosynthetic pathway.

The target of ammonium regulation is not clear. It was suggested by Bearder (1983) that the dehydrogenation of GA_4 to GA_7 is reduced by nitrogen excess, which inhibits the GA_4 1,2-dehydrogenase preferentially. To determine the influence of a high ammonium concentration on the activity of this enzyme, we used a mutant blocked at the last step in GA_3 biosynthesis, which therefore produced mostly GA_7. If the 1,2-GA_4 dehydrogenase is the major site of NH_4^+ inhibition, the GA_4:GA_7 ratio should increase sharply because of the accumulation of GA_4. Although the GA_4:GA_7 ratio rose slightly, the reduction of GA_7 formation was not associated with an increase in GA_4, the precursor of GA_7. Therefore, the 1,2-GA_4 dehydrogenase is probably not the major NH_4^+-sensitive enzyme of gibberellin synthesis in *G. fujikuroi*. The target of ammonium inhibition must lie earlier in the biosynthetic pathway, because of the decrease in all the analysed gibberellins by $(NH_4)_2SO_4$.

Bu'Lock et al (1974) studied the regulation of the biosynthesis of two different secondary metabolites—GA_3 and the polyketide bikaverin (a red pigment)—in relation to the nitrogen concentration. In batch culture with progressively more acute nitrogen limitation, bikaverin synthesis is initiated first, and then GA_3 synthesis follows. It is interesting that this series of events can be reproduced in chemostat cultures growing at different rates. These authors (Bu'Lock et al 1974) concluded that the gibberellin pathway is under the same type of overall regulation by growth rate as bikaverin synthesis,

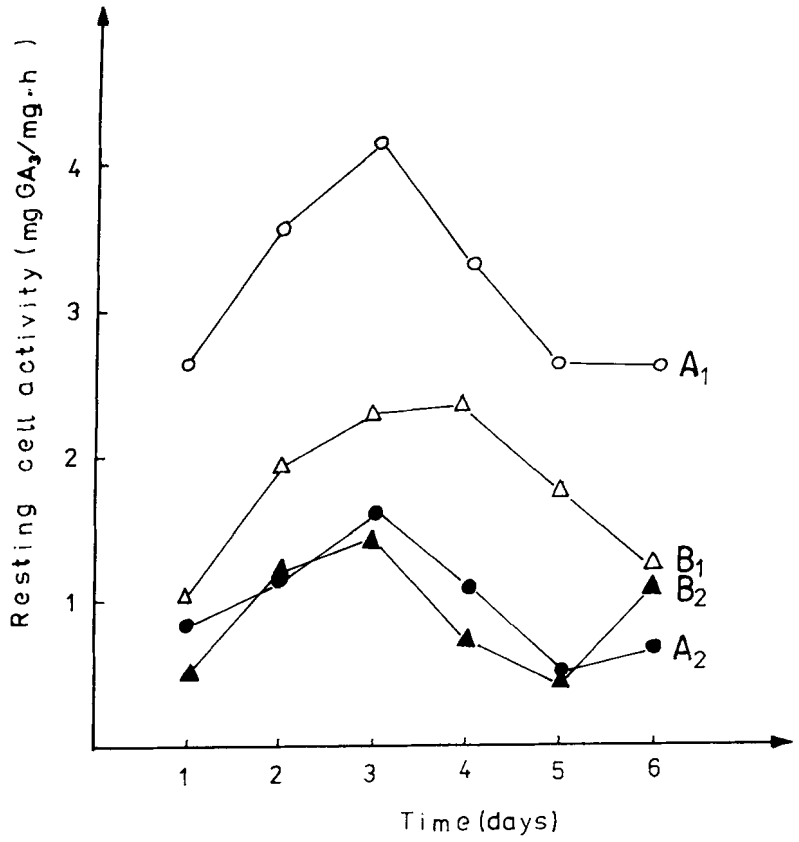

FIG. 4. Resting cell activity of cultures previously grown on (A) 19 mM or (B) 38 mM $(NH_4)_2SO_4$ in a resting cell system with 38 mM $(NH_4)_2SO_4$ or without $(NH_4)_2SO_4$. ○, △, resting cell system without $(NH_4)_2SO_4$; ●, ▲, resting cell system with $(NH_4)_2SO_4$.

but that GA synthesis begins at a lower level of limited nitrogen and a corresponding lower growth rate. In their opinion, a drastic decrease in growth rate caused by phosphate or other limitations should give the same onset of GA biosynthesis as nitrogen limitation. To test this suggestion, we examined the influence of phosphate limitation on the formation of gibberellin under conditions of nitrogen excess. Large amounts of ammonium prevent marked GA formation, although the growth rate is reduced drastically by phosphate limitation. Therefore, nitrogen controls gibberellin biosynthesis specifically. On the other hand, there are some new results on regulatory genes in *Fusarium* strains which control the biosynthesis of secondary metabolites produced on different pathways. Bu'Lock & De Gomez (1990) have described a pleiotropic

regulatory mutation in *Fusarium graminearum* which prevents the expression of the zearalenone pathway and also prevents the expression of the isoprenoid pathway leading to trichothecene mycotoxins. Up to now, we have not been able to find a similar regulatory mutant which has at the same time lost the ability to produce bikaverins and gibberellins.

Finally, it should be pointed out that the distinction between trophophase (the period of growth and primary metabolism) and idiophase (the phase of secondary metabolism) is true for gibberellin production only under laboratory conditions. Zak (1976) suggested that gibberellins may play a role in the initial infection of the host plant. In contrast to submerged cultivation, where the main production of gibberellins begins only after fungal growth has ceased, gibberellin production in infected plants occurs during the exponential phase of fungal growth. Gibberellin-produing strains of *F. moniliforme* produced significantly more biomass than non-producing strains. Therefore, gibberellin formation seems to be the phytopathogenic principle of this fungus.

Interestingly, the main steps in the gibberellin biosynthetic pathway are identical or quite similar in plants and in the fungus. Chapman & Ragan (1980) have suggested that the occurrence of this pathway in *Gibberella* can be considered as an example of gene transfer from plants to the fungus. The close association of *G. fujikuroi* with host plants may have provided an environment for natural genetic engineering.

An answer to this interesting question can be found only by cloning the specific genes for gibberellin biosynthesis from the fungus and comparing them with the analogous genes of the host plant.

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DISCUSSION

Demain: Dr Brückner, you told us that a large number of secondary metabolites are made by *Gibberella fujikuroi*. Does that mean that there are many different strains of this organism, each of which make some of these metabolites, or that any particular strain makes many gibberellins, *and* makes all the other secondary compounds that are found?

Brückner: There are many strains of the species *G. fujikuroi*, and each of the strains will make some of these different compounds, but probably not all. We use one strain which was isolated from rice. It makes gibberellin, and it also makes carotenoids and bikaverin.

Davies: Is the formation of these other compounds also regulated by light?

Brückner: Light regulates especially the isoprenone compounds, which include the carotenoids, as well as the gibberellins.

Baldwin: You mentioned that glucose inhibits the terpene pathway through HMG-CoA reductase. Is that actually proved, at the enzyme level?

Brückner: No; this is only a suggestion, that when mevalonic acid overcomes the inhibitory effect of glucose, the inhibition must be the result of the lowering of mevalonic acid production with glucose, and so only when we give mevalonic acid can we overcome the glucose effect. This means that this key enzyme must be affected by glucose. But we don't know if it is an inhibition of the activity of this enzyme, or, for instance, an influence on the *de novo* synthesis of HMG-CoA reductase.

Demain: Of course, this suggestion would only be valid if, when you add mevalonic acid to the oil fermentation, you do *not* see a stimulatory effect on gibberellin production.

Brückner: There is really no effect on gibberellin yield; but the productivity is very high and it's not possible to find a further increase with mevalonic acid.

Cane: You want to be cautious here, because the main pathway for mevalonic acid metabolism will be for the production of ergosterol in this organism, at least before the resting stage. Unlike cholesterol production, which is closely linked to HMG-CoA reductase, in studies on the formation of metabolites such as sesquiterpene phytoalexins in tobacco plant tissue cultures, there is no simple correlation between HMG-CoA reductase activity and terpenoid phytoalexin production (Vögeli & Chappell 1988, Chappell et al 1991). It could be that the branch point is later on—for example, in the induction of the geranylgeranyl diphosphate synthase, or the kaurene cyclase. This has to be determined experimentally.

I am interested in the stimulation of metabolite production by light, because there are no light-harvesting organelles in the fungus, and only a very small number of enzymic activities are known to be driven by light. I am curious about the mechanism by which an organism like this would respond to light.

Turner: There are one or two examples now in fungi of light regulation. A gene for carotenoid biosynthesis in *Neurospora crassa* is regulated by blue light (Nelson et al 1989), and in *Aspergillus nidulans* light is required for conidiation (Mooney & Yager 1990). In *Aspergillus*, although the light receptor hasn't been identified, conidiation requires red light and is inhibited by far red light. It is interesting that a mutation was put into *Aspergillus nidulans* by geneticists so that it wouldn't need light for sporulation, the velvet mutation. Exactly how the velvet gene is involved in this light response is not known.

Chater: There has been molecular analysis of light induction of carotenoid synthesis in the myxobacteria, by a group in northern Spain (Martínez-Laborda et al 1990) and by Dave Hodgson at Warwick University. This may be a good model for looking at light effects.

Bu'Lock: There is a very marked division of opinion from laboratory to laboratory as to whether we should investigate light effects! The experimental set-ups required to demonstrate and define light effects are exceedingly tedious; they are also very fruitful, within certain limitations. Very often, people from the more botanical sciences are the ones prepared to do these things. Others of us decide just to leave the light on and to refrain from investigating light effects!

The same tends to happen in relation to trace metals. You can decide to follow Weinberg's approach and investigate trace metal effects in every system you look at, which is a minefield and can give you quite extraordinary effects. My view is that we are not going to investigate trace metal effects; use the tap! Providing you don't move from one part of the country to another, you are

all right. But in fact, occasionally you cannot escape profound trace metal effects. One such is an effect on bikaverin synthesis, which, in unpublished work from our laboratory, was shown to be highly zinc determined. But once you enter into the world of trace metal effects, you have to you start a whole lot of experiments that are very difficult to finish. So, most of the time, we tend not to investigate them.

Dr Brückner, is there any evidence for a phosphate limitation of gibberellin production?

Brückner: No, we have no evidence. We compared two conditions, excess of phosphate and limited phosphate. Under conditions of excess, we see normal growth; with a low phosphate concentration (0.1 g/l KH_2PO_4) we saw a reduced growth rate, but nevertheless the rate of production of gibberellin was very low in both cases; this was because of the excess of ammonium ion in this study. Therefore, gibberellin formation is under the specific control of nitrogen but not of phosphate or of growth rate.

Chater: Dr Brückner, is there any information about whether the plant in which *G. fujikuroi* grows contributes any inducers of gibberellin synthesis?

Brückner: I know of only one publication on that. Dr Zak studied the growth of non-producing strains and a gibberellin-producing strain on rice plants. He found that non-producing strains have a lower growth rate and that the gibberellin-producing strain was at an advantage and grew faster and produced GA_3 from the beginning of exponential growth (Zak 1976). This is in contrast to laboratory conditions, where GA formation starts only after growth has been finished.

Chater: Could this be because the plant provides a non-specific inducer?

Brückner: It is possible. On the other hand, I think that the nutrient compounds are in limited concentrations and no inhibitory effects can occur.

Chater: Or there might also be a *specific* response of the plant to the fungus. Dr Brückner, are the experiments you are describing with a strain that has already been developed as a high producing strain, or a wild-type strain?

Brückner: These experiments are done with a wild-type strain.

Piepersberg: I am wondering whether the mutants blocked in gibberellic acid biosynthesis would infect the rice plant, and whether you have any idea of what gibberellin does in the infection process?

Brückner: I think the blocked mutant should grow, but at a lower rate. A specific case is the so-called 'bakanae' disease of rice. Only the gibberellic acid-positive organisms produce the disease. The blocked mutants do not cause the super-elongation of rice seedlings. The pathogenic process of this disease involves the production of gibberellic acid.

Piepersberg: But the mutants can nevertheless still infect the natural host?

Brückner: Yes; we know a lot of diseases of *Fusarium* strains on plants, not only because of gibberellin production, but also simply because of fungal growth on the plants, such as in tomatoes and other crops. But I cannot say whether

gibberellin plays a role in these other diseases, in plants such as lupins, tomatoes or carnations. I don't think so. In general, *Fusarium* strains proved to be aggressive phytopathogens for different reasons—for example, because of the formation of phytotoxins like fusaric acid.

Demain: Has anyone taken a fungal mutant that cannot make gibberellic acid and tried to infect a plant with it?

Brückner: Yes, and in both cases, mutant and wild-type, the organism can grow in the plant, but at a different rate. Gibberellic acid producers grow faster, which suggests that GA helps the infection of plants; because of the GA_3-induced elongation of the cells, maybe the fungus can more easily invade the plant tissues.

Chater: A possible experiment would be to feed a plant with gibberellic acid, after infecting it with a non-producing mutant; then you might get the full growth rate.

Davies: Does gibberellic acid enter the plant, through some lesion on the plant surface?

Brückner: Yes. In the plant, the gibberellins as natural plant hormones are in low concentrations, but when you give GA_3 from outside, at higher concentrations, this causes the hormonal balance to be destroyed.

Davies: In relation to that point, you suggested that GA_4 and GA_7 intermediates are the more agriculturally interesting. Why is that?

Brückner: There are several new publications on the functions of GA_4 and GA_7 and there may be some other effects apart from those of gibberellic acid—for instance, in the production of seeds by conifers, or in the treatment with GA_4 and GA_7 to reduce russet on 'golden delicious' apples. Another important field of application of GA_4 and GA_7 is the induction of male flower formation in monoecious and gynoecious cucumbers.

Demain: There are different agricultural effects of the different gibberellins. I have always thought of gibberellins as the equivalent of steroids in animals, but the problem has been that except for GA_3 (gibberellic acid), the gibberellins have not been available for large-scale testing in agriculture. As I recall from my industrial experience, the mixture of GA_4 and GA_7 has been shown to have specific effects on plants, not shown by gibberellic acid. But then, as you say, there are very many different gibberellins, perhaps as many as 79 known ones, and they are not available for individual testing.

Turner: The repression of production rates by glucose is reminiscent of penicillin production in *Penicillium* or *Aspergillus*. Nobody has really resolved this glucose repression effect. In *Penicillium* and *Aspergillus*, as in your organism, it's not an absolute repression. Glucose slows down the production, but will not stop it. I wonder to what extent one can separate a glucose effect from the simultaneous effect of altered growth rates, so it's actually that glucose is *speeding up* the growth, and the faster growth leads to the repression of production?

Bu'Lock: That is the usual impression. The key observation is that with *Penicillium chrysogenum* you can get round the inhibitory glucose effect by substituting a rate-determining slow feed with glucose for a big batch with a non-glucose source supplied from the beginning. To go beyond that and ask what is the actual mechanism of that glucose effect is more difficult, and there has been comparatively little work. People tend to be empirical and to get round it by using soluble starch or something like that as their carbon source.

Turner: We have been able to show that this 50% or so inhibition of penicillin production by glucose in *Aspergillus nidulans* does act at the level of the genes (Brakhage et al 1992). You can make reporter genes (by fusing the promoter of the ACV synthase and the IPNS, to appropriate reporter genes). With glucose, a pattern of a slow increase in production of the reporter gene product occurs, about 50% down on the growth on lactose. So there seems to be an effect from glucose acting at the gene level, again not an absolute effect.

In *A. nidulans* there are mutations called *cre*, which are catabolite-repression mutations. Where glucose acts on the other genes in a clearer way, such as on the gene for isocitrate lyase, when it will repress enzyme formation, or alcohol dehydrogenase, if you introduce a *creA* mutation, there is no repression with glucose. But one of these *cre* mutations that we tried doesn't affect the glucose repression (or whatever this repression is) in the *Aspergillus* antibiotic regulation mechanism; it doesn't seem to be mediated by the normal catabolite-repression system.

Demain: You mention two enzymes (IPNS and ACVS) that you made fusions with. Did they both behave in the same way?

Turner: We found that the IPNS is affected by glucose, whereas the ACVS is not.

Demain: In cephalosporin production we see an inhibitory effect of glucose but there is no glucose effect on the formation of ACVS, yet a strong one on IPNS formation. I don't want to extrapolate to *Penicillium* (because there is some indirect evidence obtained by others [Revilla et al 1986] of a glucose effect on *Penicillium* ACVS formation), but there is absolutely no repressive effect on ACVS synthesis in *Cephalosporium acremonium* (*Acremonium chrysogenum*) or *Streptomyces clavuligerus*. We do find a curious type of inhibition by glucose of the *activity* of ACVS, which may or may not have physiological importance (Zhang & Demain 1991). In cell-free extracts, it can be explained by a competition for ATP between the ACVS and the early steps of glycolysis; this inhibition can be reversed by increasing the ATP concentration. With regard to repression by glucose, it's mainly acting on later enzymes in the β-lactam pathway, especially the expandase enzyme. This enzyme is usually the most sensitive in terms of glucose repression, in both *Cephalosporium* and *Streptomyces*. The IPNS is also susceptible. It would be very interesting to know what the mechanisms are, but there has been almost no work on the basic mechanism of these glucose effects. In other organisms—for example, in the

studies of George Jones (1985) on phenoxazinone synthase of actinomycin synthesis—there is an effect of glucose on transcription. With the β-lactams, there have been no molecular studies, until this work that Professor Turner mentions.

Haslam: Could I ask a rather more general question? One of the themes that is apparent in this area of the biosynthesis of secondary metabolites is that acetic acid is a prime building block; it's the major building block for many sorts of compounds. To what extent can one ascribe the effects of various additives in changing gibberellin production, which is a very specific example, to changes in the energy state of the cell that is producing these compounds? Acetyl-CoA is a key point in the generation of energy in the cell; to what extent are all these effects simply reflecting the partitioning of acetyl-CoA in different directions?

Bu'Lock: We certainly have some ideas on this. We have examined a polyketide-type product and an isoprenoid-type product in several different systems. The system in which we have done most work is the gibberellin-producing *Gibberella fujikuroi* (Bu'Lock et al 1974). If you use a nitrogen-limited medium with a fairly high K_S value (we used glycine as nitrogen source, which has a much higher K_S than ammonium), you can run a continuous culture which doesn't make either compound; you can also run such a culture at a lower growth rate which makes, predominantly, and within analytical limits, the bikaverin polyketide; and you need to reduce the growth rate even lower to get full expression of gibberellin synthesis. Gibberellin and bikaverin are using the same acetyl-CoA pool; but which of the two you get seems to depend, not on the magnitude of that pool, but on the competition between enzymes doing different things to it. That is also my impression from less recent definitive work with the two biosynthetic routes from acetyl-CoA in *Fusarium graminearum*, to the polyketide zearalenone and the isoprenoid trichothecenes.

Brückner: Yes, and we have studied the competition for mevalonic acid. When we make mutants which cannot produce carotenoids, they sometimes produce a higher yield of gibberellins, because the entire mevalonate pool goes into the gibberellin pathway. The effect of acetate is difficult to study because of pH effects and transport problems. It's not possible to get good results, but mevalonic acid acts as an effective precursor and increases the gibberellin yield.

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