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Biochemistry

Metabolite gene regulation of the L-arabinose operon in *Escherichia coli* with indoleacetic acid and other indole derivatives

(circumvention of cyclic AMP/positive control by indole derivatives)

ELLIS L. KLINE, CAROLYN S. BROWN, VYTAS BANKAITIS, DAVID C. MONTEFIORI, AND KEN CRAIG

Department of Microbiology and Department of Biochemistry, Clemson University, Clemson, South Carolina 29631

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ABSTRACT **The ability of indole derivatives to facilitate RNA polymerase transcription of the L-arabinose operon in *Escherichia coli* was shown to require the catabolite activator protein (CAP) as well as the *araC* gene product. Adenosine 3′,5′-monophosphate (cAMP) was not obligatory for *araBAD* transcription when the cells were grown in the presence of 1 mM indole-3-acetic acid or in the presence of indole-3-acetamide, indole-3-propionic acid, indole-3-butyric acid, or 5-hydroxyindole-3-acetic acid. However, these indole derivatives were unable to circumvent the cAMP requirement for the induction of the lactose and the maltose operons. Catabolite repression occurred when glucose was added to cells grown in the presence of L-arabinose and 1 mM indoleacetic acid or 1 mM cAMP. This effect was reversed at higher concentrations of indoleacetic acid or cAMP. The induction and the catabolite repression phenomena were quantitated by measuring the differential rate of synthesis of L-arabinose isomerase (the *araA* gene product). These results indicated that indole metabolites from various living systems may regulate gene expression and may be involved in "metabolite gene regulation."**

The induction of the L-arabinose regulon—*araBAD*, *araE*, and *araF*—in *Escherichia coli* requires the protein product (P2) of the *araC* gene, the catabolite activator protein (CAP), and adenosine 3′,5′-monophosphate (cAMP) (1–8). Hence, mutants with an inactive product for the *crp gene* (the gene coding for CAP), the *araC* gene, or the *cya* gene (the gene coding for adenylate cyclase) are unable to utilize L-arabinose as a carbon source.

The existence of *araC*^{c} mutants that can circumvent the requirement for cAMP in the induction of the L-arabinose operon (2) and the complexity of various regulatory interlocks (9–11) led to the idea that low molecular weight metabolites other than the ubiquitous cAMP and guanosine 3′,5′-monophosphate might function at the genetic level in cell regulation. Recently, we demonstrated with *cya* deletion strains of *E. coli* (12) that specific concentrations of imidazoleacetic acid, a compound structurally related to the amino acid histidine, could circumvent the necessity for cAMP in the induction of the *araBAD* structural genes. In addition, preliminary evidence indicated that a metabolite of tryptophan, indole-3-acetic acid (IAA), could function in the "metabolite gene regulation" of eukaryotic cells and various genera of bacteria.*

In this paper we have investigated the ability of tryptophan and its naturally occurring metabolites IAA, 5-hydroxyindole-3-acetic acid (5-OH-IAA), indole-3-propionic acid (IPA), indole-3-butyric acid (IBA), indole-3-acetamide (IAM), indole-3-ethanol, indole-3-acetaldehyde, 5-hydroxytryptamine, and other indole derivatives to initiate transcription of the L-arabinose structural genes. The kinetics of induction and catabolite repression of the *araBAD* operon in the presence of IAA have also been examined.

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MATERIALS AND METHODS

Chemicals. All chemicals used were purchased from Sigma except for sulfuric acid, carbazole, and reagent salts, which were purchased from Fisher.

Bacterial Strains. The origins and genotypes of *E. coli* strains employed in this study are given in Table 1. The L-arabinose operon from *E. coli* B/r was placed into the *E. coli* K-12 genetic background by phage P1 cotransduction with *pdxA*.

Growth Media. The various minimal and complex media used have been described (1).

Transductions. P1 transduction experiments were performed by the method of Gross and Englesberg (7).

Mutagenesis Screen. Indole and its derivatives were screened for their mutagenic potential according to the method Ames *et al.* (14), using the *Salmonella typhimurium* strains TA98, TA1538, and TA100 and mutagens described in ref. 14 as controls. Reversion analysis was carried out (12).

Plate Induction Test. The induction screening test was performed in the dark by the method of Kline *et al.* (12).

Test of Ability of Indole Compounds To Serve as a Carbon Source. Each of the indole compounds studied was added as the sole carbon source to a minimal base medium plate. These plates were then streaked with KC14, KC10, and KC8 (the latter two both carry *cya*-2$^{\Delta}$) and examined for growth after 72 hr.

L-Arabinose Isomerase Assay. Preparation of extracts (12), measurement of the differential rate of enzyme expression (15), and the L-arabinose isomerase assay (12) have been described.

Growth Kinetics and Catabolite Repression. A phenotypically characterized *cya*$^{-}$ single colony isolate was grown in the absence of light and growth was monitored as described (12). In the catabolite repression studies, KC13 (*cya*-2$^{\Delta}$) cells were grown in 1500-ml flasks containing 700 ml of minimal medium supplied with 0.4% L-arabinose and 1 mM cAMP or 1 mM IAA. At an OD_{660} of 0.35, the cultures were split. Half the culture was allowed to grow without treatment as it had prior to the split. To the other portion, glucose was added at a final concentration of 0.5%. Growth was subsequently followed spectrophotometrically at 660 nm in both cultures. To quan-

Abbreviations: CAP, catabolite activator protein; cAMP, adenosine 3′,5′-monophosphate; IAA, indole-3-acetic acid; 5-OH-IAA, 5-hydroxy-IAA; IPA, indole-3-propionic acid; IBA, indole-3-butyric acid; IAM, indole-3-acetamide.

* Kline, E. L., Bankaitis, V., Brown C. S., Montefiori, D. C. & Craig, K. (1979) *Abstracts of the Annual Meeting of the American Society for Microbiology*, K104, P15, H40.

Table 1. *E. coli* strains

Strains		Characteristics*	Source†
B/r	UP1007	Wild type	E. Englesberg
K-12	LS853	*his-85* λ^- *trpA9605 trpR55 cya-2*$^{\Delta}$‡	B. J. Bachmann
B/r	DC1	*ara-leu-1119*$^{\Delta}$ *pro-1* $T6^r$ *try-10 dau-5* Str^r	D. P. Kessler
B/r	SB2074	*ara-leu-1170+1235*$^{\Delta}$ *dau-5* Str^r	D. P. Kessler
B/r	SB5000	*leuB1 dau-5*	Nancy Lee
B/r	SB5004	*leuB1 dau-5 arg-2*	Et_2SO_4-induced Arg^- SB5000
B/r	DC7	*araC766*$^{\Delta}$ *dau-5 arg-2*	P1*bt* SB1085 × SB5004
B/r	DC71	*leuB1* Mal^+ λ^s	D. P. Kessler
B/r	DC74	*ara-leu-1170+1238*$^{\Delta}$ *dau-5* Str^r Mal^+ λ^s	P1*bt* DC71 × SB2074
K-12	CU356	*gal*$^-$ *ilvDAC115*$^{\Delta}$ *leu-455*	EtMes-induced Leu^- CU344 (7)
K-12	CU359	*gal*$^-$ *ilvDAC115*$^{\Delta}$ *pdxA204*	P1*bt* WB1473 × CU356
K-12	KC2	*gal*$^-$ *ilvDAC115*$^{\Delta}$ *araC766*$^{\Delta}$	P1*bt* 7 × CU359
K-12	KC4	*gal*$^-$ *ilvDAC115*$^{\Delta}$ *ara-leu-1170+1238*	P1*bt* 74 × CU359
K-12	KC7	*gal*$^-$ *ilvDAC115*$^{\Delta}$	P1*bt* DC1 × CU359
K-12	KC8	*gal*$^-$ *araC766*$^{\Delta}$ *cya-2*$^{\Delta}$	P1*kc* KC13 × KC2
K-12	KC10	*gal*$^-$ *ara-leu1170+1238*$^{\Delta}$ *cya-2*$^{\Delta}$	P1*kc* LS853 × KC4
K-12	KC13	*gal*$^-$ *cya-2*$^{\Delta}$	P1*kc* LS853 × KC7
K-12	KC14	*gal*$^-$	P1*kc* LS853 × KC7
K-12	KC15	*gal*$^-$ *araC766*$^{\Delta}$	P1*kc* KC13 × KC2
B/r	SB5616	*ara-leu-1119*$^{\Delta}$ *dau-5 cya-4 crp*$^-$ Str^r	G. Wilcox
B/r	EB1078	*dau-5 cya-4 crp*$^-$ Str^r	P1*bt* UP1007 × SB5616
B/r	DC455	*ara-leu-1101*$^{\Delta}$ *araD59* Str^r	D. P. Kessler
B/r	EB1079	*dau-5* Str^r	P1*bt* EB1078 × Dc455

* Auxotrophic requirements: *ara-leu-1170+1238*, leucine; *ara-leu-1119*, leucine; *ara-leu-1101*, leucine; *leuB1*, leucine; *his*$^-$, histidine; *trp*$^-$, tryptophan; *pro*$^-$, proline; *ilv*$^-$, isoleucine and valine, *pdxA204*, arginine and uracil. Abbreviations: *dau-5*, D-arabinose negative; Str^r, streptomycin resistant; λ^s, *E. coli* B/r sensitive to λ infection; $T6^r$, phage T6 resistant; *cya*$^-$, adenylate cyclase deficient; *crp*$^-$, negative for cAMP receptor protein; Mal^+, maltose positive; *gal*$^-$, D-galactose negative; *ara*$^-$, L-arabinose negative; $^{\Delta}$, deletion.

† Et_2SO_4, diethyl sulfate; Arg^-, arginine requiring; EtMes, ethyl methanesulfonate; Leu^-, leucine requiring.

‡ *cya-2* is a deletion, called DE36 by the Coli Genetic Stock Center and *cya283* by Brickman *et al.* (13).

titate the extent of induction of the L-arabinose operon, samples were removed from both flasks at specified times and cells were compared with respect to their L-arabinose isomerase activities. Relief of catabolite repression by cAMP or IAA was determined as follows: A 500-ml culture of KC13 was grown initially in L-arabinose minimal medium supplemented with either 1 mM IAA or 1 mM cAMP. Duplicate samples were collected when OD_{660} reached 0.35. The remainder was divided into four 100-ml cultures supplemented with 0.4% L-arabinose, 0.5% D-glucose, and either IAA or cAMP (as in the initial culture) at 0, 1, 3, or 5 mM. Duplicate samples were obtained from each culture after 2 hr and L-arabinose isomerase was assayed.

Experimentation. All plate analyses, growth kinetic experiments, enzyme kinetic experiments, and catabolite repression experiments were performed at least three times.

Protein Determination. The protein concentrations of the cell extracts were determined by the Lowry method (16).

RESULTS

Effect of Indole Derivatives on the Induction of *ara, mal, lac,* and *leu* Operons. In the bacterial system used, if a compound elicited growth of KC13 (*cya-2* deletion strain) on L-arabinose, maltose, or lactose as the sole carbon source and did not serve as a carbon source itself, it was considered to be a metabolite gene regulation MGR (1) molecule that could circumvent the cAMP necessity in the induction of the L-arabinose, the maltose, or the lactose operon, respectively. The ability of several indole derivatives to circumvent the cAMP necessity in the induction of the *araBAD* operon is demonstrated in Table 2. As indicated, IAA, 5-OH-IAA, IPA, IBA, and IAM were effective in promoting expression of the L-arabinose structural genes. The necessity for CAP and the product of the *araC* gene (P2) for induction by these indole derivatives was demonstrated by the inability of any of these compounds to stimulate L-arabinose utilization in a *cya-2*$^{\Delta}$ strain carrying an *araC* gene lesion (KC15) or a *cya*$^-$ strain carrying a *crp* lesion (EB1078). When any one of the indole derivatives was added to EB1078 (*cya-4, crp*$^-$) in the presence of cAMP, no growth was observed (Table 2), suggesting that induction by these compounds was dependent on CAP even in the presence of cAMP. These observations indicated that these metabolites could act at the level of transcription.

Although the indole derivatives could circumvent the necessity for cAMP in the initiation of transcription of the L-arabinose system, they were unable to function effectively in the induction of the lactose or the maltose systems (Table 3). They were also unable to derepress the leucine biosynthetic operon and thus could not produce an L-arabinose-positive response in strain KC10, a strain with an *ara-leu* fusion that places the *araBAD* genes under leucine control (9).

Inability of Indoles To Serve as Carbon Sources. The inability of indole derivatives used in this study to serve as a carbon source was demonstrated by the lack of growth of KC14, KC8, or KC10 on minimal medium supplemented with any one of these compounds.

Mutagenicity. The possibility of indole and indole derivatives being mutagenic was examined by the Ames test (14), using methyl methanesulfonate, 9-aminoacridine, and 2-aminofluorene as control mutagens. Indole and its derivatives proved unable to revert frameshift or point mutations in *Salmonella*, indicating they have no mutagenic activity.

Growth Kinetics in the Presence of IAA. Exogenous IAA at a concentration of 1 mM could promote maximal growth of KC13 (*cya-2*$^{\Delta}$) in liquid cultures containing L-arabinose as the sole carbon source (Fig. 1*A*). These results substantiated the

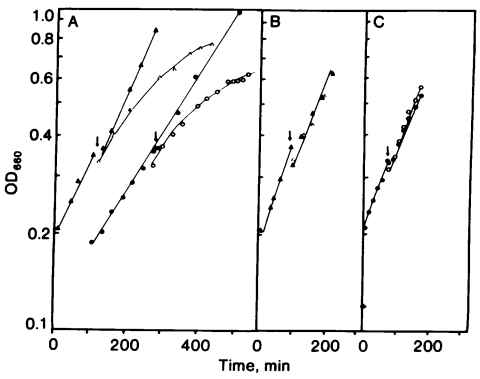

FIG. 1. Growth of KC13 (*cya-2*$^{\Delta}$) (*A*) and KC14 (isogenic wild type) (*B* and *C*) on L-arabinose minimal medium in the presence (▲) or absence (△) of 1 mM exogenous cAMP, and in the presence (●) or absence (○) of 1 mM exogenous IAA. All cultures were maintained at 37°C in a gyratory shaking water bath; arrows indicate shift of medium.

Table 2. Ability of indole and indole metabolites to circumvent the cAMP requirement in L-arabinose operon induction

Strain	Pertinent markers	Compound* tested with dilution plate method†															
		cAMP	I	IAA	5-OH-IAA	IE	IAL	IPA	IBA	IAM	IN	IAE	Trp	5-OH-Trp	TRA	Trp-NH_2	5-OH-TRA
KC14	Wild type *gal*$^-$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
KC13	*cya-2*$^\Delta$ *gal*$^-$	+	−	+	+	−	−	+	+	+	−	−	−	−	−	−	−
KC8	*cya-2*$^\Delta$ *araC766*$^\Delta$ *gal*$^-$	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KC10	*cya-2*$^\Delta$ *ara-leu-1170+1238*$^\Delta$ *gal*$^-$	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
EB1078	*cya-4 crp*$^-$	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KC15	*araC766*$^\Delta$ *gal*$^-$	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DC455	*ara-leu-1101*$^\Delta$	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
EB1078	*cya-4 crp*$^-$ (cAMP supplement)‡	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Abbreviations: I, indole; IE, indole-2-ethanol; IAL, indole-3-acetaldehyde; IN, indole-3-nitrite; IAE, indole-3-acetic acid ethyl ester; Trp, L-tryptophan; 5-OH-Trp, 5-hydroxytryptophan; Trp-NH_2, L-tryptophanamide; TRA, tryptamine; 5-OH-TRA, 5-hydroxytryptamine.

† Screening method for *ara* operon induction described previously (12). A − indicates no growth after 48 hr; a +, strong growth of all dilution streaks after 48 hr.

‡ cAMP was at 1 mM in L-arabinose minimal medium.

plate analysis, which indicated that this compound could circumvent the necessity for cAMP in the induction of transcription of the L-arabinose operon (Tables 2 and 3). IAA or cAMP dilution profile analysis of KC13 inoculated into L-arabinose minimal medium containing either cAMP or IAA at exogenous concentrations of 5, 4, 3, 2, 1, 0.75, and 0.1 mM and 10, 1, 0.1, and 0.01 μM demonstrated an ability for growth at 5–0.75 mM cAMP or 4–0.75 mM IAA, with 1 mM exogenous IAA or cAMP being most effective in growth stimulation of KC13. The growth rate of KC13 in the presence of 1 mM exogenous IAA was somewhat slower than the growth rate in the presence of 1 mM exogenous cAMP (Fig. 1*A*). A shift of KC13 from medium containing either 1 mM cAMP or 1 mM IAA to medium without the positive effector resulted in greatly reduced growth rates within 1.5 hr. Neither cAMP nor IAA at exogenous concentrations of 1 mM significantly affected the growth rate of the isogenic wild-type (*cya*$^+$) strain, KC14 (Fig. 1 *B* and *C*). It should be noted, however, that 5 mM exogenous cAMP reduced the growth rate of KC14 in minimal medium and that even higher concentrations of cAMP or a concentration of 5 mM IAA were apparently bacteriostatic.

Effect of IAA on the Differential Rate of Synthesis of L-Arabinose Isomerase (*araA* Gene Product). Comparison of the differential rates of enzyme synthesis of L-arabinose isomerase in KC13 grown in the presence of 1 mM IAA or 1 mM cAMP (both of which allow for maximal growth rate of KC13 on L-arabinose minimal medium) as the positive effector indicated a significantly reduced rate of synthesis in the presence of IAA (Fig. 2*A*). Synthesis of L-arabinose isomerase continued for approximately 1.5 hr after shift of either of these cultures to medium without effector and then sharply decreased. This abrupt decrease may be due to cessation of transcription concomitant with degradation or inactivation of L-arabinose isomerase. The induction of the L-arabinose operon in the isogenic wild-type strain, KC14, was unaffected by either 1 mM IAA or 1 mM cAMP (Fig. 2 *B* and *C*).

Catabolite Repression of *araBAD* by Glucose in cAMP- or IAA-Induced Cells. When D-glucose was added to a culture of KC13 growing on L-arabinose in the presence of 1 mM cAMP, the cAMP-mediated induction of the L-arabinose isomerase was catabolite repressed (Fig. 3). Similar effects were seen when glucose was added to KC13 cells grown on L-arabinose in the presence of 1 mM IAA.

The generation time of KC13 growing in the presence of L-arabinose and 1 mM IAA decreased from 185 min to 125 min upon the addition of glucose to a growing culture. When glucose was added to this organism growing in the presence of L-arabinose plus 1 mM cAMP, the generation time decreased from 110 min to 95 min.

Because cAMP at elevated concentrations has been observed to reverse catabolite repression of a culture growing in glucose (17), the ability of increased concentrations of IAA to reverse catabolite repression was determined. When 3 mM IAA was present upon glucose addition to a growing L-arabinose, IAA-induced culture, there was an apparent decrease in the catabolite repression response; this was also the case with cAMP (Table 4).

DISCUSSION

In higher organisms, many hormones exert their actions via the intracellular "second messenger," cAMP (18, 19). Subsequent to fluctuations in intracellular concentrations of cAMP [or cyclic GMP, or both (20, 21)], dramatic changes occur in cellular

Table 3. Phenotypic character elicited by indole metabolites that circumvent the cAMP requirements

Strain	Pertinent markers	Growth on carbon source								
		D-Lactose			L-Arabinose			D-Maltose		
		cAMP	Indole metabolites	H_2O	cAMP	Indole metabolites	H_2O	cAMP	Indole metabolites	H_2O
KC14	Wild-type *gal*$^-$	+	+	+	+	+	+	+	+	+
KC13	*cya-2*$^\Delta$ *gal*$^-$	+	−	−	+	+	−	+	−	−
KC10	*cya-2*$^\Delta$ *ara-leu-1170+1238*$^\Delta$ *gal*$^-$	+	−	−	−	−	−	+	−	−
KC8	*cya-2*$^\Delta$ *araC766*$^\Delta$ *gal*$^-$	+	−	−	−	−	−	+	−	−
EB1078	*cya-4 crp*$^-$	−	−	−	−	−	−	−	−	−
DC455	*ara-leu-1101*$^\Delta$	+	+	+	−	−	−	+	+	+

"Indole metabolites" refers to IAA, 5-OH-IAA, IPA, IBA, and IAM, which give identical growth responses.

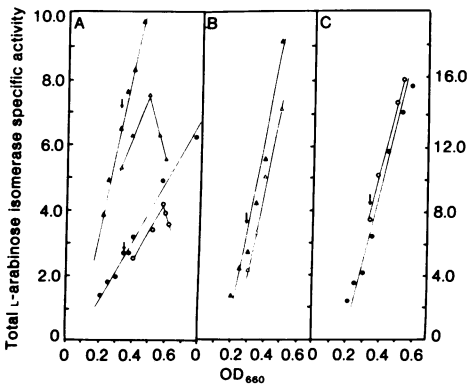

FIG. 2. Differential rates of L-arabinose isomerase synthesis of KC13 (*cya-2*$^{\Delta}$) (*A*) and KC14 (isogenic wild type) (*B* and *C*) grown in L-arabinose minimal medium in the presence (▲) or absence (△) of 1 mM exogenous cAMP, and in the presence (●) or absence (○) of 1 mM exogenous IAA. Units for *B* and *C* are on the right ordinate. Total L-arabinose isomerase specific activity = μmol of L-ribulose formed per hr per mg of protein. The shift points from media containing positive effector (cAMP or IAA) to media without positive effector are indicated by arrows.

metabolism. In prokaryotes, the compound cAMP also plays a role in the induction of certain catabolite-repressible operons such as L-arabinose, lactose, galactose, and maltose (22–24). In all the latter systems, cAMP is proposed to interact with a protein molecule, CAP, to function in gene regulation. The L-arabinose system requires an additional factor, the *araC* gene product (P2), to initiate transcription.

Several indole derivatives were observed in this study to circumvent the necessity for cAMP in eliciting gene expression in the L-arabinose system of *E. coli*. In addition, glucose was observed to catabolite repress the *araBAD* operon in KC13 when either cAMP or IAA was present. This effect could be reversed if higher concentrations of cAMP (17) or IAA were present (Table 4). Both CAP and the *araC* gene product were required for these molecules to function as positive effectors.

The L-arabinose, the maltose, and the lactose operons all require cAMP–CAP for induction. However, the indole derivatives that were found to circumvent the necessity for cAMP in eliciting gene expression in the L-arabinose operon were unable to do so in the lactose and the maltose operons. This selective expression would suggest that there are differences between the L-arabinose transcription initiation site and the sites in the lactose and maltose operons. Indeed, it has been shown by DNA nucleotide sequence analysis that there are regions of dissimilarity at the promotor locus in the L-arabinose operon (25) and the lactose operon (26). This observation is further supported by the additional requirement of the *araC* gene product for L-arabinose induction (6).

The compounds IAA, IPA, IBA, and IAM are all known to

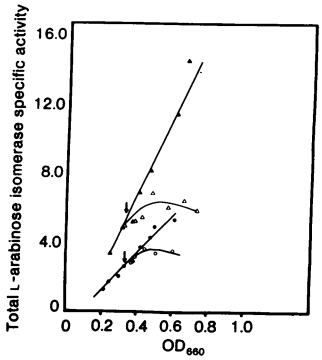

FIG. 3. Catabolite effect of 0.5% D-glucose on the total L-arabinose specific activity (μmol of L-ribulose formed per hr per mg of protein) of KC13 (*cya-2*$^{\Delta}$) growing in L-arabinose minimal medium supplemented with 1 mM cAMP (●, ○) or 1 mM IAA (▲, △). ● and ▲, No glucose present; ○ and △, after addition of glucose. The points of glucose addition are indicated by arrows.

Table 4. Relief of catabolite repression with cAMP or IAA

Growth condition	IAA or cAMP, mM	L-Arabinose isomerase specific activity*	
		cAMP	IAA
L-Arabinose	1	17.5	11.7
L-Arabinose + D-glucose	1	9.6	7.5
L-Arabinose + D-glucose	3	20.9	14.2
L-Arabinose + D-glucose	5	21.1	NT

NT, not testable due to the extremely slow growth rate.
* μmol of L-ribulose formed per hr per mg of protein.

provide auxin activity in higher plants and as such regulate a number of growth-related processes (27–32). IAA has also been shown to affect enzyme expression in eukaryotic cells and to increase the production of various enzymes in eukaryotic and prokaryotic systems.* It has been reported (33) that IPA can function at the genetic level in *E. coli* by derepressing the *trp* operon. Thus, at least one indole metabolite (IPA) used in this study can function in the regulation of both positively and negatively controlled operons in *E. coli*. IBA has been found to affect cell growth in rats (34), to have a hypoglycemic effect in rats (35), and to promote and accelerate root formation of plant clippings (36). IAM also functions as a plant hormone (32).

In many animal and plant species, 5-OH-IAA is a natural metabolite of 5-hydroxytryptamine (serotonin) (37). Although 5-OH-IAA itself has not previously been reported to have any regulatory role, 5-hydroxytryptamine is a neutrotransmitter (37), has been implicated in behavioral phenotypes (38–44), and is a precursor of the hormone melatonin (45). The aldehyde derived from this amine is also considered to have biological activity (46–49).

Comparisons of the structures of the indole derivatives that induced the L-arabinose operon with those that are inoperative in the system indicated that a 2- to 4-carbon alkyl side chain was required for activity. The parent compound, indole, could not initiate transcription of *araBAD*. Four out of five of the effective compounds had alkyl carboxylic acid chains attached to the indole ring structure. An amino group on the propionic acid side chain (tryptophan, 5-hydroxytryptophan) resulted in a molecule that was inactive. The amide of tryptophan was also ineffective in *araBAD* induction. A hydroxyl group at the 5 position of the ring of IAA (5-OH-IAA) had no apparent effect on the ability of the compound to induce. However, a hydroxyl group on the alkyl side chain (indole-3-ethanol) made the molecule inactive in the inductive process. Although IAA was a positive effector, the reduction product (indole-3-acetaldehyde) was not functional in this capacity. The neutral amide of indoleacetic acid (IAM) was functional but the indole-3-acetic acid ethyl ester was inactive. The decarboxylated amino acids (tryptamine and 5-hydroxytryptamine) were unable to induce the L-arabinose operon. An indole ring with nitrite at the 3 position was also unable to elicit L-arabinose utilization. Thus, it appears that indole with short (2- to 4-carbon) alkyl carboxylic acid side chains (or neutral amide derivatives of these acids) have potential as cAMP substitutes in the induction of the L-arabinose operon in *E. coli*.

IAA or cAMP at an exogenous concentration of 1 mM had no apparent effect on the growth rate or the differential rate and extent of induction of the *araBAD* operon in the *cya*$^{+}$ background. However, in KC13 (*cya-2*$^{\Delta}$) the growth rate and rate and extent of induction of the *araBAD* operon was less in the presence of exogenous 1 mM IAA than in the presence of exogenous 1 mM cAMP. It is possible that IAA was not transported into the cell as well as cAMP.

Glucose elicited catabolite repression in KC13 growing on

L-arabinose and 1 mM IAA. This repression was overcome when higher concentrations of IAA were present, as was the case with cAMP (17). These observations demonstrated that changes in cAMP concentrations were not obligatory for glucose-mediated catabolite repression responses in *E. coli*. Perhaps glucose or some other metabolite can directly or indirectly affect the concentration of certain factors (possibly P2) that function in the induction of the L-arabinose operon. Alternatively, glucose or other metabolites may compete with cAMP (or IAA)–CAP or with other necessary factors for DNA initiation sites.

From studies in our laboratory in both prokaryotic and eukaryotic cells, it is becoming increasingly evident that naturally occurring metabolites other than cAMP can function at the genetic level to control cell activity, a concept that we have named "metabolite gene regulation" (12). Why indole and imidazole derivatives (12) can circumvent cAMP necessity in the induction of the L-arabinose operon is not completely understood. With regard to possible interactions of small molecules with nucleic acids, it is of interest that Hendry and coworkers have demonstrated with molecular models that histidine, tryphophan, and IAA as well as cAMP (refs. 50–52; L. B. Hendry, personal communication) can intercalate into double-stranded sequences of RNA and DNA and stress hydrogen bonding. It has been reported that polyhydroxylic compounds affect transcription, presumably by disrupting hydrogen bonding in the DNA molecule (51, 52). This disruption of hydrogen bonding may be a way in which a selective region on the DNA is enhanced for recognition by RNA polymerase.

Because the sequence of molecular events in the interaction of cAMP or the cAMP–CAP complex with the DNA is not completely resolved, one can only speculate about the nature of these interactions. For instance, one might envision cAMP (or IAA) bound to an exposed rather than a buried region of CAP, still allowing intercalation of the cAMP (IAA) into the DNA as demonstrated by the models of L. B. Hendry *et al.* (personal communication). Or cAMP (IAA) might induce a conformational change in the DNA that would subsequently allow the formation of a DNA–cAMP (IAA)–CAP complex. Although we favor the interpretation that IAA in these studies is functioning in a manner similar to cAMP, these data do not rule out the possibility that IAA is affecting synthesis or activation of some other component that, in turn, induces the L-arabinose operon.

The observations presented in this study illustrate the complexities of cell regulation and the need for multifaceted approaches in the investigation of cellular metabolism in order to comprehend the metabolic state of different types of cells and possible mechanisms of cellular differentiation.

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