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Evidence of Multiple Regulatory Functions for the PtsN (IIA^{Ntr}) Protein of *Pseudomonas putida*

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The *ptsN* gene of *Pseudomonas putida* encodes IIA^{Ntr}, a protein of the phosphoenol pyruvate:sugar phosphotransferase (PTS) system which is required for the C source inhibition of the σ^{54}-dependent promoter *Pu* of the TOL (toluate degradation) plasmid pWW0. Using two-dimensional gel electrophoresis, we have examined the effect of *ptsN* disruption on the general expression pattern of *P. putida*. To this end, cells were grown in the presence or absence of glucose, and a 1,117-spot subset of the *P. putida* proteome was used as a reference for comparisons. Among all gene products whose expression was lowered by this carbon source (247 spots [about 22%]), only 6 behaved as *Pu* (i.e., were depressed in the *ptsN* background). This evidenced only a minor role for IIA^{Ntr} in the extensive inhibition of gene expression in *P. putida* caused by glucose. However, the same experiments revealed a large incidence of glucose-independent effects brought about by the *ptsN* mutation. As many as 108 spots (ca. 9% of the cell products analyzed) were influenced, positively or negatively, by the loss of IIA^{Ntr}. By matching this pattern with that of an *rpoN*::ΩKm strain of *P. putida*, which lacks the σ^{54} protein, we judge that most proteins whose expression was affected by *ptsN* were unrelated to the alternative sigma factor. These data suggest a role of IIA^{Ntr} as a general regulator, independent of the presence of repressive carbon sources and not limited to σ^{54}-dependent genes.

The *Pu* promoter of *Pseudomonas putida* drives transcription of the upper operon of the TOL plasmid pWW0, which makes this strain capable of using toluene, *m*-xylene, or *p*-xylene as the only source of carbon and energy (2). Besides being induced by pathway substrates (24), this σ^{54}-dependent promoter is repressed by threefold in the presence of certain carbon sources such as glucose or gluconate (3, 11). We have recently reported that the loss of the *ptsN* gene appears to relieve this C-source-dependent inhibition (3), an event that can be genetically distinguished from other down-regulation effects caused by fast growth (5). *ptsN* is included in the so-called *rpoN* gene cluster, which determines not only the sigma factor σ^{54}, but also four more downstream genes (3, 15). In particular, *ptsN* encodes a type II enzyme (termed IIA^{Ntr}) of the phosphoenol pyruvate:sugar phosphotransferase (PTS) system (3, 15), which is a complex and very branched group of phosphotransfer proteins involved in controlling the intake of certain carbohydrates and other regulatory functions (reviewed in reference 21).

Homologues of *ptsN* have also been found adjacent to *rpoN* in various other gram-negative species, including *Escherichia coli*, *Klebsiella pneumoniae*, *Caulobacter crescentus*, *Rhizobium meliloti*, and *Pseudomonas aeruginosa* (12, 13, 18, 19, 22). *ptsN* mutants of *K. pneumoniae* (in which *ptsN* was originally called ORF152) displayed an increased activity of the σ^{54}-dependent promoter *PnifH* (18). On the contrary, the loss of the equivalent *ptsN* (ORF2) in *P. aeruginosa* did not affect the activity of its σ^{54} promoters for pili and flagellin genes (13). Furthermore, some (but not all) of the *Caulobacter* and *Rhizobium* σ^{54} systems tested became less active upon the loss of *ptsN* (12, 19). A *ptsN* mutant of *E. coli* displayed certain incompatibilities between C and N sources—typically glucose and alanine (22). In addition, this mutation also suppressed a temperature-sensitive allele of *era*, a gene encoding an essential GTPase of unknown function (22). These observations, made with various systems, are not easy to reconcile. On one hand, they suggest the existence of a specific molecular pathway for physiological coregulation of some σ^{54} promoters in which IIA^{Ntr} is a key intermediate. On the other hand, IIA^{Ntr} might also be involved in more general metabolic activities, such as coordination of C and N metabolism (22, 23, 25). This is plausible, since the PTS system also participates in a group of regulatory processes (21) in a fashion dependent on the availability of adequate carbohydrates in the external medium. These act as a drain of high-energy phosphate, which determines the accumulation or the depletion of phosphorylated intermediates, which have the ability to interact with and modify the activity of many other cell products (21). In this regard, there is genetic evidence that a phosphorylated form of IIA^{Ntr} mediates the repressive effect of glucose on the *Pu* promoter (3).

With these premises, we set out to explore the role of the *ptsN* gene of *P. putida* in the general pattern of protein expression. To this end, we resorted to two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) analysis of proteins from *P. putida* cells bearing a *ptsN* disruption, grown in the presence or the absence of a repressive carbon source such as glucose. As shown below, 2D electrophoresis allowed us to measure the simultaneous influence of IIA^{Ntr} on the levels of a large number of gene products. Our data indicate that *ptsN* is involved in the expression of a considerable share of the entire *P. putida* proteome, either activating or inhibiting the outcome of approximately 9% of the gene products analyzed. Interestingly, most of these effects were unrelated to the presence of glucose in the medium. Comparison of the protein

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patterns of the *ptsN* strain versus those of an *rpoN* mutant indicated that IIA^{Ntr} modulates expression of both σ^{54}-dependent and σ^{54}-independent products.

MATERIALS AND METHODS

Bacterial strains. *P. putida* MAD2 is a tellurite-resistant derivative of *P. putida* KT2442 bearing a chromosomal *Pu-lacZ* fusion along with an *xylR* allele named *xylRΔA* (8). The loss of the N-terminal A-domain endows XylR with a constitutive activity, independent of inducer (*m*-xylene) addition, but still responsive to down-regulation by C source (3, 4). Strain *P. putida* MAD2 *ptsN*::Km is a derivative in which the *ptsN* gene has been disrupted in the 53rd codon by the insertion of two copies of a promoterless Km^r cassette (3). The $ptsN^+$ plasmid pJM154 is a derivative of broad-host-range vector pJPS9 inserted with a *Pst*I fragment spanning the genomic region of *P. putida* that carries *ptsN*, but excluding the genes adjacent to the *rpoN* cluster (3). The *P. putida rpoN*::ΩKm strain was constructed by Köhler et al. (14).

Culture conditions and other general methods. Cells were grown at 30°C in M9 minimal medium (20) with all amino acids added (except methionine) (M9-AA) at the concentrations reported by Davis et al. (7). Where indicated, the cultures were supplemented with 10 mM glucose. The excess of Casamino Acids in the medium equaled growth rates and avoided effects related to the stringent response (31).

2D electrophoresis and analysis of expression patterns. Cultures inoculated with the strains under scrutiny were grown up to an optical density at 600 nm (OD_{600}) of ~1.5. At that point, 1-ml aliquots of each sample were pulse-labeled with 45 μCi of [^{35}S]methionine (specific activity, 1,000 Ci/mmol) for 10 min and then chased with cold methionine for 3 min. Cells were spun down and resuspended in 60 μl of sodium dodecyl sulfate (SDS)-β-mercaptoethanol buffer (0.3% SDS, 5% β-mercaptoethanol, 50 mM Tris-Cl [pH 8]) and boiled for 2 min. Samples were then treated 30 min on ice with a DNase-RNase solution (final concentration, 15 mg of DNase I per ml, 75 mg of RNase A per ml, 1 mM $MgCl_2$), and then 240 μl of lysis buffer (6 M urea, 2 M thiourea, 4% CHAPS {3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate}, 1% IPG buffer [pH 3 to 10], 2 mM TCEP-HCl) was added. Samples were then clarified by ultracentrifugation. A total of 1.5×10^6 cpm, previously diluted to 350 μl in a mixture containing 6 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer (pH 4 to 7), and 1 mM TCEP-HCl, was applied by rehydration of IPG strips (nominal pH gradient of 4 to 7, 18-cm length; Pharmacia Biotech, Uppsala, Sweden). Samples were focused by stepwise increase of the voltage as follows: 30 V for 6 h, 60 V for 6 h, 500 V for 30 min, 1,000 V for 30 min, and 1,000 to 8,000 V for 30 min. Gels were then subjected during the next 30 min to a linear increase from 8,000 V to 60,000 V. After isoelectric focusing separation, strips were equilibrated twice for 15 min with a mixture containing 50 mM Tris-HCl (pH 8.8), 6 M urea, 30% glycerol, 2% SDS, and traces of bromophenol blue. The first equilibration step contained 1% dithiothreitol, and the second had 4% iodoacetamide added. The 2D SDS-PAGE was performed with 1-mm-thick, 16 by 15-cm, 12.5% homogeneous polyacrylamide gels. Electrophoresis was carried out overnight at 5°C at a constant current of 5 mA. Gels were then dried, and radioactive signals were detected in a Storm aparatus (Pharmacia Biotech). Duplicate gels were run and analyzed for every genetic background and for the growth conditions described in this article, which included strains *P. putida* MAD2, *P. putida* MAD2 *ptsN*::Km, *P. putida* MAD2 *ptsN*::Km (pJM154), *P. putida* KT2442, and *P. putida* KT2442 *rpoN*::ΩKm, each grown in the presence or absence of glucose. ImageMaster v 3.01 software (Pharmacia Biotech) was used for spot detection and detailed analysis.

RESULTS

Global repression of gene expression caused by glucose in $ptsN^+$ and *ptsN* strains of *P. putida*. Since the *ptsN* gene product has been correlated with glucose repression of the *Pu* promoter of the TOL plasmid (3, 5), we set out to investigate the extent of this regulatory role of *ptsN*. To this end, we grew the wild-type *P. putida* MAD2 and *P. putida* MAD2 *ptsN*::Km strains in M9 medium supplemented with all of the amino acids except methionine and with or without 10 mM glucose added. The cultures were then labeled with [^{35}S]methionine, and their protein extracts were run in a 2D gel electrophoresis system. As a control, β-galactosidase assays carried out in parallel showed that under the conditions of the experiment, the *Pu-lacZ* fusion was indeed repressed in the presence of glucose and derepressed in its absence (not shown). The resulting gels are shown in Fig. 1. Quantitative analysis of the 1,117 most prominent spots revealed well-defined changes in the intensity of many distinct polypeptides in response to the presence of glucose in the wild-type background. Expression of 247 spots (22%) out of all the proteins displayed in the 2D gels were reduced by ≥2-fold in extracts from the wild-type, $ptsN^+$ *P. putida* MAD2 cultures with glucose added.

When the intensity of these glucose-repressible spots was examined in extracts from the *ptsN* counterpart also grown in the presence of glucose, only 12 proteins appeared to have lost down-regulation by the carbohydrate. In these cases, the levels in the presence of the sugar equaled or exceeded those of the $ptsN^+$ wild-type strain (Fig. 2). Moreover, when the *P. putida* MAD2 *ptsN*::Km mutant strain was transformed with plasmid pJM154 (which carries the wild-type *ptsN* allele), 6 of the 12 spots that were not repressed by glucose in the mutant reverted to being down-regulated by the sugar. Since the previously observed effect of glucose on activity of the *Pu* promoter of the TOL plasmid was restored upon complementation (3), the failure to revert the *ptsN* phenotype for half of the spots could be due to partial polar effects of the Km insertion in downstream genes (see Discussion). In any case, the modest proportion of proteins that behaved similarly to those expressed through *Pu* indicated that *ptsN* was not a mayor player in the extensive inhibition of gene expression caused by glucose on *P. putida*.

Surveying connections between IIA^{Ntr}-dependent and σ^{54}-dependent regulation. Most functions reported for *ptsN* and its encoded product IIA^{Ntr} in vivo are related to up-regulation or down-regulation of σ^{54}-dependent systems (3, 12, 18, 19). In view of the data presented above, the next issue was whether the changes brought about by the disruption of *ptsN* were in all cases dependent on σ^{54}. To examine this point, we carried out the same type of 2D gel analysis with extracts of strain *P. putida* KT2442 *rpoN*::ΩKm grown under conditions equal to those used before. An important feature of this *rpoN*::ΩKm strain is that the insertion of a Km interposon (14) within the *rpoN* gene has a strong polar effect on expression of the genes downstream of the operon, as detected in Western blots with anti-IIA^{Ntr} serum (not shown). The reference *P. putida rpoN*::ΩKm strain constructed by Köhler et al. (14) and used in this work thus fails to express not only *rpoN*, but also *ptsN* (and probably the further downstream genes of the *rpoN* gene cluster) (3). The six protein spots whose inhibition by glucose was unequivocally mediated by *ptsN* were examined in such an *rpoN*::ΩKm strain (Fig. 2). Interestingly, only one of them (protein spot 1500) was absent, in both the presence and absence of glucose, suggesting that its expression was indeed dependent on σ^{54} (or other genes of the *rpoN* cluster) (3). Two other proteins of the group (spots 60 and 91) behaved in the *rpoN*::ΩKm strain like they did in the *ptsN*::Km mutant (i.e., they were fully expressed regardless of the presence of the C source), but in an apparent σ^{54}-independent fashion. Finally, the other three spots (276, 1424, and 1428) behaved basically like they did in the wild-type strain. Such a compensation for the loss of IIA^{Ntr} by the lack of σ^{54} and/or other genes of the *rpoN* cluster is not easy to explain. One possibility is that some genes that behave this way could be expressed through multiple promoters such that transcription is only σ^{54} dependent in

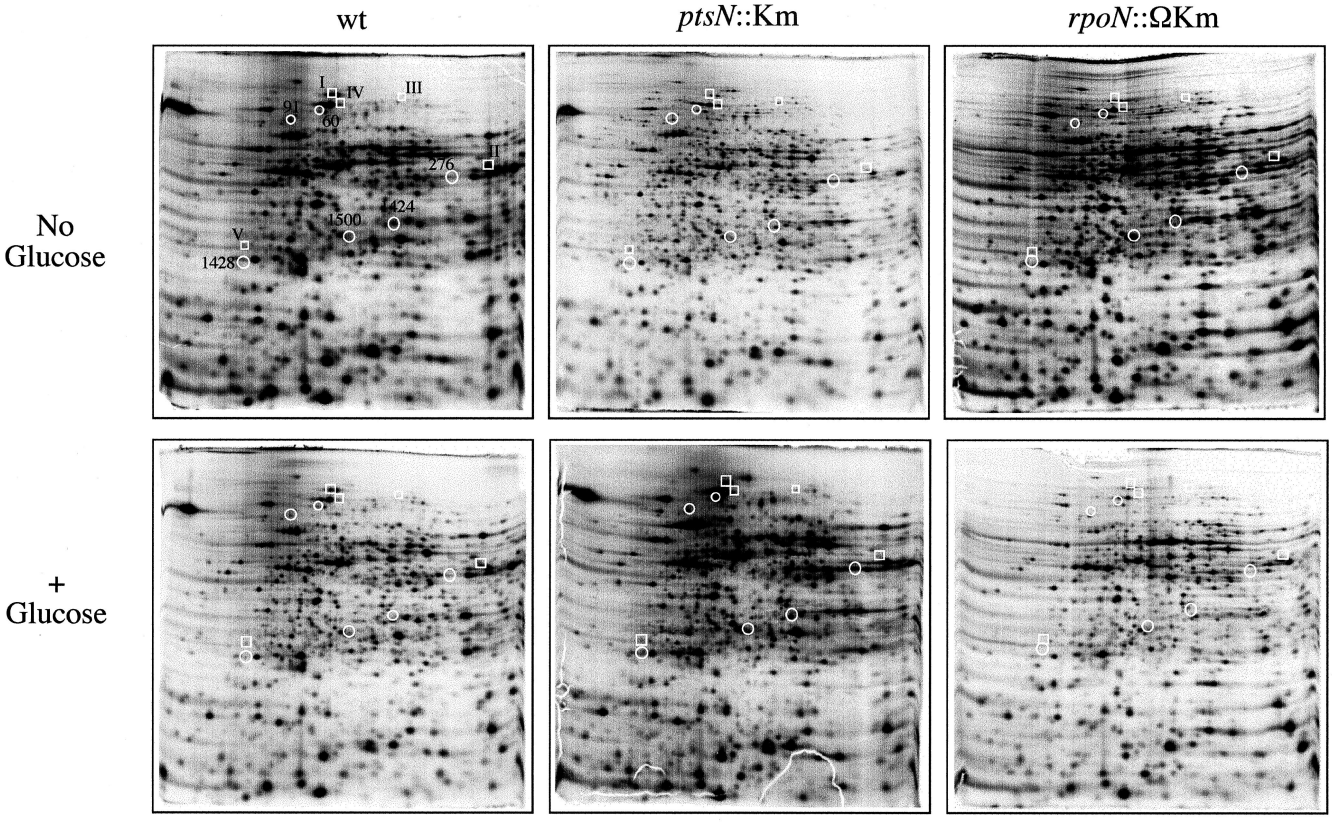

FIG. 1. 2D gels of protein extracts from *P. putida* MAD2 (wt), *P. putida* MAD2 *ptsN*::Km, and *P. putida rpoN*::ΩKm. Cultures of each strain were grown in M9-AA medium (supplemented or not with 10 mM glucose, as indicated) until early stationary phase (OD_{600} of ~1.5). Cultures were then labeled with [^{35}S]methionine as explained in Materials and Methods. Protein extracts were first electrofocused in a pH 4 to 7 gradient and then run across a 12.5% denaturing PAGE system. The autoradiographs of a subset of dried 2D gel results used for the scanning are shown here. A selection of spots whose intensity changes depending on the strain is indicated for reference: boxed spots (types I to V) correspond to proteins affected by the lack of *ptsN* (further examined in Fig. 3); circled spots (affected by glucose) coincide with the proteins whose expression is shown in Fig. 2.

the presence of glucose (e.g., the activator protein required is only active under glucose-supplemented conditions). However, as discussed above, because of the polar effect of the *rpoN*::ΩKm insertion, the compensation for the loss of *ptsN* may in some cases be unrelated to σ^{54}.

Glucose-independent effects caused by the loss of *ptsN* on global gene expression. Further analysis of the 2D gels revealed a significant number of additional changes between the wild-type and *ptsN* extracts that were entirely independent of the presence or absence of glucose in the medium (Fig. 3). Up to 134 proteins out of the 1,117 spots analyzed were clearly down-regulated by ≥5-fold in the *ptsN*-negative background. In the other direction, at least 250 proteins were distinctively overexpressed in the mutant. Both events (repression and overproduction of different sets of proteins in the *ptsN* mutant) occurred in both glucose-containing and glucose-free media. These observations clearly indicated that the regulatory consequences of the loss of *ptsN* are not restricted to the presence of glucose. In addition, they suggest that depending on the specific gene product, IIA^{Ntr} may act as a positive or a negative factor in a regulatory cascade.

Among the 134 spots whose expression was reduced ≥5-fold in the *ptsN*-negative genetic background (i.e., which required IIA^{Ntr} for full expression), 48 recovered normal levels upon complementation of the mutant strain with plasmid pJM154 (Fig. 3, types 1 and 2). When the same 48 proteins were inspected in the *rpoN*::ΩKm strain lacking σ^{54}, 10 of them were present at levels like those found on the *ptsN*-less background or lower (type 1 in Fig. 3). Whether or not these proteins require σ^{54} for expression cannot be ascertained with this experimental setup, since, as mentioned above, the polar effect of the ΩKm insertion also inhibits expression of the *ptsN* gene and the rest of the open reading frames (ORFs) of the *rpoN* cluster (3).

The expression levels of the remaining 38 spots in the *rpoN*::ΩKm strains were comparable to those of the wild type (Fig. 3, type 2), thereby indicating that they were fully independent of σ^{54} for expression. The conclusion of these experiments is that the bulk of the gene products which require an intact IIA^{Ntr} protein for expression under various growth conditions are unrelated to σ^{54}.

Contrary to the subset of proteins whose expression needed *ptsN*, 219 spots had an increased intensity in the *ptsN* strain compared to that of the wild-type *P. putida* strain, thus suggesting that IIA^{Ntr} had a negative rather than a positive effect on their expression. Only 54 of these changes were reversed to normal in the *P. putida* MAD2 *ptsN*::Km(pJM154) complemented strain. Out of this whole of 54 proteins genuinely

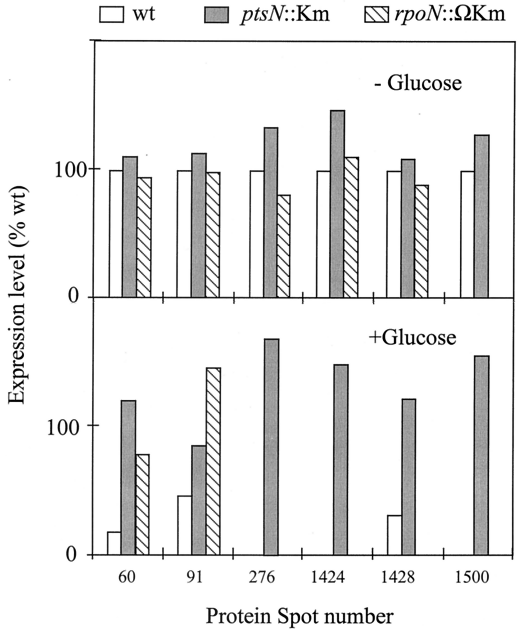

FIG. 2. Expression of selected glucose-repressible spots in *P. putida* MAD2 (wt), *P. putida* MAD2 *ptsN*::Km, and *P. putida rpoN*::ΩKm. Cultures of each of these strains were grown, labeled, and resolved in 2D gels as explained in the legend to Fig. 1. Expression levels are plotted as a percentage of those of the wild-type strain in the absence of glucose. An expression level of zero indicates levels below detection by our experimental setup. The six spots under scrutiny (numbered 60, 91, 276, 1424, 1428, and 1500 in Fig. 1) are down-regulated by glucose only if the wild-type *ptsN* gene is present, but they are fully expressed in the *ptsN*-negative mutant, regardless of the added C source. Expression of these six proteins whose inhibition by glucose was dependent on IIA^{Ntr} was reexamined in the proteome of the σ^{54}-negative strain. Note that spots 60 and 91 are derepressed in the *rpoN*::ΩKm strain as they are in the *ptsN*::Km background, thereby indicating that their down-regulation by C source is IIA^{Ntr} dependent but σ^{54} independent (see text for explanation).

repressed by IIA^{Ntr}, 5 were entirely missing in the *rpoN*::ΩKm strain, thus indicating that their expression required σ^{54} either directly or indirectly (Fig. 3, type 3). Among the remaining 49 proteins derepressed in the *ptsN*::Km strain, 19 turned out to be derepressed as well in the *rpoN*::ΩKm strain, probably due to the polar effects of the ΩKm insertion discussed above. However, the rest (30 proteins) behaved in the *rpoN*-negative strain like they did in the wild-type *P. putida* strain, thus providing another clue that σ^{54} plays a role in IIA^{Ntr}-mediated regulatory events (Fig. 3, types 4 and 5).

A side result of this set of experiments was to realize the importance of σ^{54} in the general expression profile of *P. putida* cells. Out of the 823 protein spots that were present in both the wild-type background and in the *ptsN* mutant under any of the conditions tested, 93 of them (i.e., close to 10% of all reference proteins) were completely lost in the *rpoN* background. These spots were missing regardless of the presence or absence of glucose and were thus considered to have a σ^{54}-dependent expression (not shown). Since the two-dimensional SDS-PAGE technology does not allow us to distinguish between direct and indirect effects, it is not possible to ascertain at this point whether σ^{54} participates directly in transcription of the genes encoding the missing protein spots. In any case, this result evidenced the extensive participation of σ^{54} in the global expression pattern of *P. putida*.

DISCUSSION

Inhibition of the expression of certain genes in response to facile carbon sources is a well known phenomenon in the microbial world (21, 28), although the mechanisms involved can be very disparate (6, 21, 28). A homologue of the *E. coli* catabolite regulatory protein (CRP) named Vfr has been described for *P. aeruginosa* and is also present in the *P. putida* genome (unpublished results). However, Vfr is involved not in C regulation but in quorum sensing (1, 27). On the other hand, a general factor encoded by the *crc* gene seems to mediate catabolite repression in both *P. aeruginosa* (17) and *P. putida* (10). However, the encoded protein belongs to a family of exonucleases, and its mechanism of action remains elusive (17), since it could mediate posttranscriptional rather than transcriptional checkpoints in expression of C-regulated genes (10). A higher level of C source regulation is controlled in *E. coli* and *Bacillus* by the PTS system, which dominates the events that trigger catabolite repression (21). In these bacteria, the PTS mediates transport of some sugars through a process that involves phosphorylation and dephosphorylation of a number of protein components in response to sugar availability (21, 28). Such protein intermediates behave as indicators of nutrient excess and general energy status. Although glucose is transported neither in *P. aeruginosa* (6, 17) nor in *P. putida* (30) by a PTS-like activity (16, 30), previous results indicate (3) that the phosphorylated form of the protein product encoded by *ptsN* (IIA^{Ntr}), a PTS type II protein, is necessary for C source repression of the σ^{54}-dependent promoter *Pu* of the TOL plasmid. As summarized in Fig. 4, the results presented in this article show that the role of *ptsN* in such a C repression of σ^{54} systems is in fact limited to just a few cases within a much wider role of IIA^{Ntr} in global expression profiles of *P. putida*.

The data described above revealed that the loss of *ptsN* affects expression of a range of proteins in a fashion entirely independent of glucose (Fig. 3). In fact, it comes as a surprise that the loss of IIA^{Ntr} influences expression of such a large number of polypeptides. Close to 10% of all of the spots analyzed in the 2D gels were found to be up- or down-regulated by IIA^{Ntr}. This could account for the diversity of phenotypes described in *ptsN* mutants of different species (12, 13, 18, 19, 22). Most of the proteins affected in the *ptsN* mutant (87 out of 102) were independent of σ^{54}, thus highlighting IIA^{Ntr} as a general regulatory factor not limited to *Pu*-like systems (25). A separate issue in this respect is the connection between σ^{54} and *ptsN* functions. As mentioned above, since the *rpoN*::ΩKm strain is a phenotypic *rpoN ptsN* double mutant, the *rpoN*::ΩKm and *ptsN*::Km strains should show partially overlapping phenotypes. However, this is not the case for a significant proportion of the spots analyzed, suggesting that some promoters may receive separate inputs through signalling pathways involving σ^{54}, IIA^{Ntr}, and even some additional genes of the *rpoN* cluster (3).

Only around 30% of all of the changes caused by the Km insertion in *ptsN* could be unequivocally traced to the lack of IIA^{Ntr}, as shown by the comparison of the mutant and the

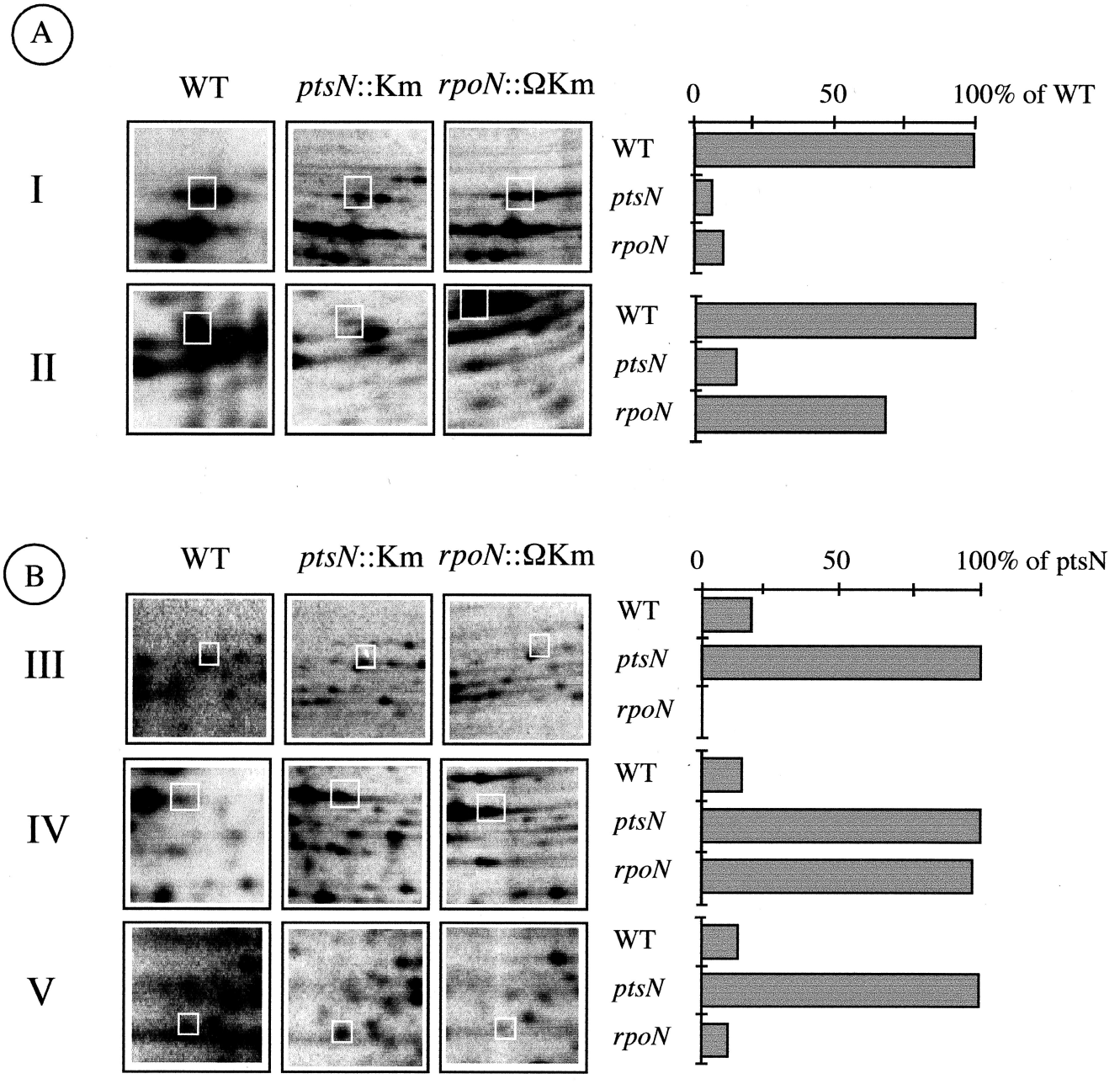

FIG. 3. Types of spots in 2D protein gels of *P. putida* KT2442 and its *ptsN*::Km and *rpoN*::ΩKm variants. The photographs to the left show representative spots from the 2D gels (numbered as shown in Fig. 1), whereas the bars to the right are a quantification of the intensities of the selected proteins that fall under the various categories, as a percentage of the full expression levels, as indicated. (A) Spots whose expression is lessened in the *ptsN*-negative strain. Proteins of this kind do appear whose expression is either reduced in both the *ptsN*::Km strain and the *rpoN*::ΩKm strain (type I) or whose level declines in the *ptsN* mutant but not in the *rpoN* strain (type II). (B) Products whose expression is increased in the *ptsN*-negative mutant. Among these, some proteins are σ^{54}-dependent products (type III), others are σ^{54} independent (type IV; expression is increased both in the *ptsN*-negative and *rpoN*-negative strains), and yet another class of spots (type V) augment their expression in the *ptsN*-negative mutant but are missing in the *rpoN* strain. Photographs and quantification values shown are from the cultures not supplemented with glucose. Similar patterns were detected in extracts from cells grown with glucose. (See text for interpretation.) WT, wild type.

strains complemented with a *ptsN*$^+$ plasmid. Although the Km insertion in *ptsN* allowed expression of downstream genes of the gene cluster (as revealed with a serum against the product of the last ORF), the levels were somewhat reduced (not shown). Some spots could therefore be controlled by one or more genes of the *rpoN* cluster other than *ptsN*. These could act in concert or independently, an issue that deserves further studies. In fact, that the same regulatory factor causes a variety of effects in expression or activity of different proteins is typical of PTS proteins (19). Examples include the phosphorylation-

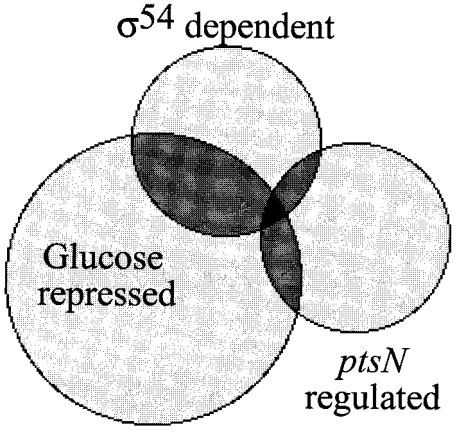

FIG. 4. Connections between IIA^{Ntr}, σ^{54}, and glucose-repressible expression in *P. putida* as revealed by 2D gel analysis of *ptsN*::Km and *rpoN*::ΩKm mutants. Among the 1,117-spot subset of the *P. putida* proteome, as many as 247 products were repressible by glucose (i.e., had expression ≥2-fold lower in the presence of the sugar), while 93 proteins were dependent on σ^{54} (i.e., were entirely missing in extracts of the *rpoN* strains under all conditions). The *ptsN*-regulated spots considered for this representation include only those whose changes (≥5-fold greater or lesser than the wild-type levels) can be complemented by a functional *ptsN* copy. The areas of the circles are approximately proportional to the number of spots included in the categories represented.

dependent ability of IIA^{Glu} to interact with a number of permeases (19) or the formation of the CcpA-HPr repressor complex in gram-positive bacteria (28). This could be the case also for IIA^{Ntr}: two forms of the factor (phosphorylated and nonphosphorylated) could specialize in regulation of different sets of proteins, in concert with other factors (such as σ^{54} itself) and other PTS components. Regardless of the specific mechanisms involved, the incidence and extension of effects linked to the *ptsN* gene make it appear to be more of a general regulator than a promoter-specific factor.

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