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Modification of a Mammalian Cell Protein in the Presence of [^{32}P-Adenylate]NAD: Evidence for ADP Ribosylation Activity Associated with *Helicobacter pylori*

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Culture filtrates from *Helicobacter pylori* promote the transfer of the radiolabel from [^{32}P-adenylate]NAD to one or more heat-labile factors within extracts prepared from several mammalian cell lines, with the predominate radiolabeled species exhibiting an apparent molecular mass of greater than 130 kDa. Our results suggest that several *H. pylori* strains release a factor that ADP-ribosylates a mammalian target protein.

Persistent human infection with the gastric pathogen *Helicobacter pylori* is a significant risk factor for the development of gastric ulcer disease (19, 14, 17), gastric adenocarcinoma (21, 23), gastric mucosa-associated lymphoid tissue lymphoma, and gastric Hodgkin's lymphoma (24, 29). The virulence mechanisms underlying *H. pylori* pathogenesis are complex and remain poorly understood (5, 6, 20). However, the discovery of several bacterial factors, including VacA (3, 10, 12, 15), urease (18, 26, 28), and the *cag* pathogenicity island (4, 22) as well as others (11), has been important for understanding the virulence strategies used by *H. pylori* during colonization and persistence. In this report, we describe evidence for the presence of ADP-ribosyltransferase activity within *H. pylori* culture filtrates. Significantly, a number of pathogenic bacteria produce ADP-ribosylating toxins or exoenzymes that specifically modify host proteins (1, 25).

Heat-labile components from both *H. pylori* culture filtrates and HeLa extracts are required to promote the incorporation of ^{32}P radiolabel from [^{32}P-adenylate]NAD into trichloracetic acid (TCA)-precipitated material. To explore the possibility that *H. pylori* may produce an ADP-ribosyltransferase that is capable of modifying a eukaryotic target, we fractionated liquid cultures of *H. pylori* 26695 (ATCC no. 700392) and monolayers of human cervical cancer epithelial-like cells (HeLa cells; ATCC no. CCL-2). Bacterial culture filtrates were prepared by centrifugation (10,000 × *g* for 20 min at 4°C) of stationary-phase *H. pylori* liquid cultures that had been cultivated at 37°C for 72 h in bisulfite-/sulfite-free Brucella broth (10 g tryptone/liter, 10 g peptamine/liter, 5 g NaCl/liter, 1 g dextrose/liter, 2 g β-cyclodextrin/liter) containing 5 μg vancomycin/ml under 10% CO_2, 10% O_2 and shaking at 200 rpm. The supernatants were concentrated by ammonium sulfate (90%) precipitation, and the ammonium sulfate pellets were resuspended and dialyzed into 50 mM Tris (pH 7.6) and 10 mM NaCl, followed by filter sterilization. HeLa cell extracts were prepared by incubating semiconfluent monolayers of cells with mammalian cell lysis buffer (MPER; Pierce, Rockford, IL), 50 mM Tris (pH 7.6), 10 mM NaCl, and a 1:100 dilution of protease inhibitor cocktail set III (AEBSF [4-{2-aminoethyl} benzenesulphonyl fluoride] [1 mM], aprotinin [0.8 μM], bestatin [50 μM], E-64 [15 μM], leupeptin [20 μM], pepstatin A [10 μM]; Calbiochem, San Diego, CA) at 25°C. After 5 min, the lysed cells were centrifuged at 20,000 × *g* at room temperature for 1 min, and the soluble extract was further clarified by filter sterilization.

H. pylori culture filtrates (1 mg protein/ml) and HeLa cell soluble extracts (1 mg protein/ml) were incubated together or separately at 25°C in the presence of either [^{32}P-adenylate]NAD or [^{3}H-nicotinamide]NAD (50 μM) in 50 mM Tris (pH 7.6), 10 mM NaCl. After 15 min, each reaction was terminated by spotting 20 μl of the mixture onto TCA (20%)-soaked, 1- by 1-in. Whatman no. 1 filter pads, followed by washing three times with ice-cold TCA (5%) and two times with ice-cold methanol to remove all soluble material, including soluble [^{32}P-adenylate]NAD or [^{3}H-nicotinamide]NAD. The dried pads were transferred to scintillation vials containing Scintiverse BD cocktail (Fisher, Hanover Park, IL), and the extent of NAD-derived radiolabel recovered with the TCA-precipitated material was scored by scintillation counting in either the ^{32}P or the ^{3}H channel using a Beckman LS 6500 scintillation counter. Because NAD is soluble, the recovery of radiolabel within the TCA-precipitated material indicates the association of some or all of the NAD molecule with macromolecules that are insoluble at an acidic pH.

These experiments revealed that reaction mixtures containing both the HeLa and *H. pylori* fractions yielded significant increases in ^{32}P radiolabel associated with the TCA-precipitated material relative to reaction mixtures containing either fraction alone (Fig. 1A). Moreover, reactions with both the *H. pylori* and the HeLa fractions yielded significantly higher levels of ^{32}P radiolabel relative to reaction mixtures in which either the *H. pylori* or the HeLa fraction had been pretreated at 90°C for 30 min (Fig. 1A). In preliminary studies, we found that, when incubated with *H. pylori* culture filtrate, only the soluble fraction from HeLa extracts (and not the insoluble membrane fractions) yielded a significant increase in ^{32}P radiolabel associated with the TCA-precipitated material (data not shown). Finally, for reaction mixtures in which bisulfite-/sulfite-free

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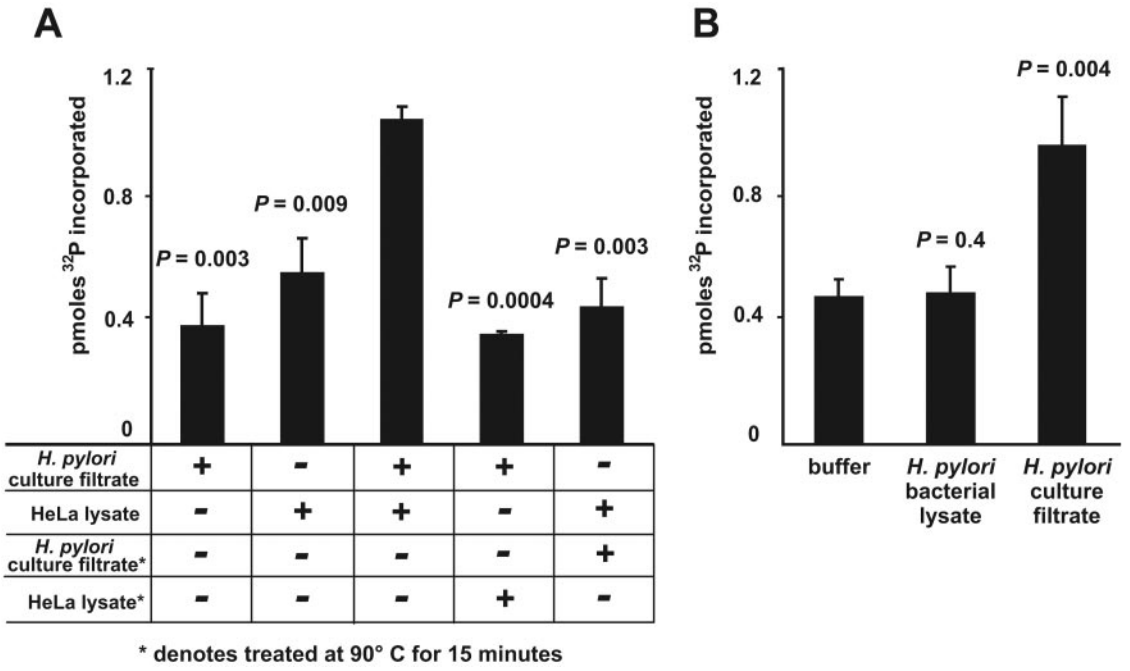

FIG. 1. Transfer of ^{32}P radiolabel from [^{32}P-adenylate]NAD to TCA-precipitated material is dependent on both *H. pylori* culture filtrate and HeLa cell extract. Combinations of fractions prepared from *H. pylori* 26695 and monolayers of HeLa cells were incubated in the presence of [^{32}P-adenylate]NAD (50 μM; Amersham Pharmacia, Piscataway, NJ) and 50 mM Tris (pH 7.6), 10 mM NaCl at 25°C. After 15 min, ^{32}P radiolabel transfer was scored by scintillation counting of ^{32}P-radiolabeled, TCA-precipitated material. (A) The reaction mixtures consisted of *H. pylori* culture filtrates (1 mg protein/ml final in reaction), which was preincubated for 30 min at either 0°C or 90°C and followed by a 10-min incubation on ice, and the soluble fraction of HeLa cell lysates (1 mg protein/ml final in reaction), which was preincubated for 30 min at either 0°C or 90°C, followed by a 10-min incubation on ice, either alone or in combination. −, absence of indicated substance; +, presence of indicated substance. (B) The reaction mixtures consisted of the soluble fraction of HeLa cell lysates (1 mg protein/ml final in reaction) with either of the *H. pylori* culture filtrates (1 mg protein/ml final in reaction), *H. pylori* bacterial lysates (1 mg protein/ml), or reaction buffer (50 mM Tris [pH 7.6], 10 mM NaCl). Each reaction was performed in triplicate, with the experiment performed at least three separate times. Error bars indicate standard deviations, and the *P* values were calculated from the Student *t* test relative to the reaction mixture containing both *H. pylori* culture filtrates and the soluble fraction of HeLa cell lysates. A *P* value of <0.05 was considered significant.

Brucella growth medium was substituted for *H. pylori* culture filtrate, we did not detect significant differences ($P = 0.4$) in ^{32}P radiolabel associated with the TCA-precipitated material relative to reactions containing HeLa extract alone (data not shown); these results rule out the possibility that a heat-labile contaminant from the *H. pylori* growth medium is alone responsible for promoting the incorporation of the NAD-derived ^{32}P radiolabel into the TCA-precipitated material in the presence of HeLa extracts. Collectively, these results indicate that heat-labile, soluble components originating from both *H. pylori* and HeLa cells are required to promote the incorporation of NAD-derived ^{32}P radiolabel into TCA-precipitated material.

To investigate whether the *H. pylori* component might also be cell associated, we tested whether whole-cell extracts prepared from stationary-phase *H. pylori* cultures could also stimulate ^{32}P radiolabel incorporation. Bacterial cells from harvested cultures were washed twice with phosphate-buffered saline (pH 7.2) and then incubated at 4°C with bacterial protein extraction reagent (BPER; Pierce, Rockford, IL) with 5 mM phenylmethylsulfonyl fluoride in 50 mM Tris (pH 7.6), 10 mM NaCl. After 4 h, the soluble and insoluble materials were fractionated by centrifugation at 20,000 × *g* at 4°C for 20 min, and the soluble extract was further clarified by filter sterilization. The lysis of bacterial cells was confirmed by monitoring the increase in soluble protein before and after treatment with BPER. In contrast to culture filtrates, *H. pylori* cell extracts did not stimulate a significant increase in ^{32}P radiolabel detected within the TCA-precipitated material (Fig. 1B) when incubated with HeLa extracts in the presence of 50 μM [^{32}P-adenylate]NAD for 15 min at 25°C. To investigate the possibility that exposure to BPER during extract preparation resulted in a loss of ^{32}P radiolabel transfer activity, we analyzed *H. pylori* extracts prepared in the absence of BPER by two alternative approaches: lysis of cells by either sonication or pneumatic press. These experiments revealed that neither sonication nor the pneumatic press yielded extracts with significantly more ^{32}P radiolabel transfer activity than those prepared with BPER (data not shown), suggesting that during preparation of *H. pylori* extracts, BPER did not cause a loss of ^{32}P radiolabel transfer activity. These data confirm that, when cultivated in vitro, the *H. pylori* component required to stimulate ^{32}P radiolabel incorporation is not cell associated but rather is found within the culture filtrate. Based on these data, we used culture filtrates throughout the remainder of this study. These results also suggest the possibility that the *H. pylori* heat-labile component may be secreted from the bacterium, although we cannot presently rule out the possibility that a fraction may be

released into the medium as a consequence of bacterial autolysis, which can occur for *H. pylori* in stationary phase (7, 27).

Notably, in experiments where [^{3}H-nicotinamide]NAD was substituted for [^{32}P-adenylate]NAD, we were unable to detect a significant increase in ^{3}H radiolabel associated with the TCA-precipitated material relative to controls lacking either *H. pylori* culture filtrate or HeLa extract (data not shown). Taken with the results described above, these data suggest that components within *H. pylori* culture filtrates and HeLa extracts together promote transfer of a phosphate-containing moiety from NAD to a protein acceptor with the concurrent release of nicotinamide; these observations are consistent with the idea of ADP ribosylation occurring within our reaction mixtures.

NAD-derived ^{32}P radiolabel associated with TCA-precipitated material as a function of *H. pylori* and HeLa fractions. Although our results indicate that *H. pylori* culture filtrate and the soluble fraction from HeLa cell extract are both important for generating the TCA-precipitated, ^{32}P-radiolabeled material, they did not reveal the source of the activity responsible for transfer of the ^{32}P radiolabel, nor did they reveal the source of the acceptor for the ^{32}P radiolabel. To further evaluate the potential sources of transfer activity and the ^{32}P radiolabel acceptor, we evaluated the dependence of ^{32}P radiolabel incorporation as a function of *H. pylori* and HeLa cell fractions. *H. pylori* culture filtrate was incubated with HeLa extract in the presence of 50 μM [^{32}P-adenylate]NAD at 25°C. The extent of ^{32}P radiolabel transfer after 15 min was scored by scintillation counting of the TCA-precipitated material within the reaction mixture. For these experiments, the concentration of either the *H. pylori* or the HeLa fraction varied, while the concentration of the other fraction was held constant. These experiments revealed that under reaction conditions not limiting for NAD, the amount of ^{32}P radiolabel incorporated into TCA-precipitated material increased in direct proportion to the concentration of HeLa extract (Fig. 2A). In contrast, when *H. pylori* culture filtrate varied, the amount of ^{32}P radiolabel increased initially but reached a maximum at concentrations greater than 1 mg protein/ml (Fig. 2B). These data indicate that under our reaction conditions, components within HeLa extract, but not *H. pylori* culture filtrate, are limiting for ^{32}P radiolabel incorporation and are consistent with the interpretation that an *H. pylori* component catalyzes the transfer of ^{32}P radiolabel from NAD to an acceptor within HeLa cell extracts.

Additional experiments revealed that the pretreatment of *H. pylori* culture filtrates with proteinase K resulted in a significant decrease in ^{32}P radiolabel incorporated into TCA-precipitated material, indicating that the factor responsible for ^{32}P radiolabel transfer was sensitive to protease action (data not shown). Moreover, ultrafiltration of *H. pylori* culture filtrate prior to analysis, using centrifugal microconcentrators with molecular mass cutoffs of 10, 30, or 50 kDa, did not result in a significant change in the amount of ^{32}P radiolabel incorporated into TCA-precipitated material (data not shown), indicating that all of the activity was retained within the microconcentrator. In contrast, when a centrifugal microconcentrator with a 100-kDa molecular mass cutoff was used, a significant reduction in the amount of ^{32}P radiolabel incorporated into TCA-precipitated material was detected, indicating a loss of activity during ultrafiltration. Taken together, these results suggest that the *H. pylori* component that catalyzes the transfer of ^{32}P radiolabel from NAD to an acceptor within HeLa cell extracts is a protein with a molecular mass of between approximately 50 and 100 kDa.

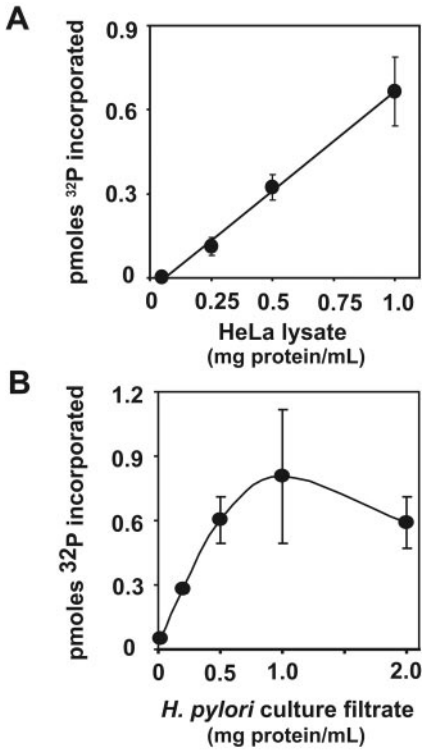

FIG. 2. The extent of ^{32}P radiolabel from ^{32}P-adenylate transferred to TCA-precipitated material as a function of both *H. pylori* culture filtrate and the soluble fraction of HeLa cell lysate. *H. pylori* culture filtrates and the soluble fraction of HeLa cell lysates were incubated at 25°C in the presence of [^{32}P-adenylate]NAD (50 μM). After 15 min, ^{32}P radiolabel transfer was scored by scintillation counting of ^{32}P-radiolabeled TCA-precipitated material. (A) The soluble fraction of *H. pylori* culture filtrate was held constant (1 mg protein/ml), while the concentration of the soluble fraction of HeLa cell lysate was variable (0.0 to 1.0 mg protein/ml). (B) The soluble fraction of HeLa cell lysate was held constant (1 mg protein/ml), while the concentration of the *H. pylori* culture filtrate was variable (0.0 to 2.0 mg protein/ml). Each reaction was performed in triplicate, with the experiment performed at least three separate times.

***H. pylori*-dependent transfer of ^{32}P radiolabel to a specific protein within the soluble fraction of mammalian cell extracts.** To evaluate the specificity of *H. pylori*-mediated transfer of the ^{32}P radiolabel from NAD, *H. pylori* culture filtrate (1 mg protein/ml) was incubated for 15 min with HeLa extract (1 mg protein/ml) in the presence of either [^{32}P-adenylate]-radiolabeled or nonradiolabeled NAD (50 μM) at 25°C. For reactions initially including nonradiolabeled NAD, [^{32}P-adenylate]NAD was added after 15 min to a final concentration of 50 μM. For reactions initially including [^{32}P-adenylate]NAD, nonradiolabeled NAD was added after 15 min to a final concentration of 50 μM. The final specific activity of [^{32}P-adenylate]NAD was identical for both reaction conditions. The extent of ^{32}P radiolabel transfer after 30 min was scored by scintillation counting of the TCA-precipitated material within the reaction mixture. These experiments revealed that the amount of ^{32}P radiolabel transferred into TCA-precipitated material was significantly

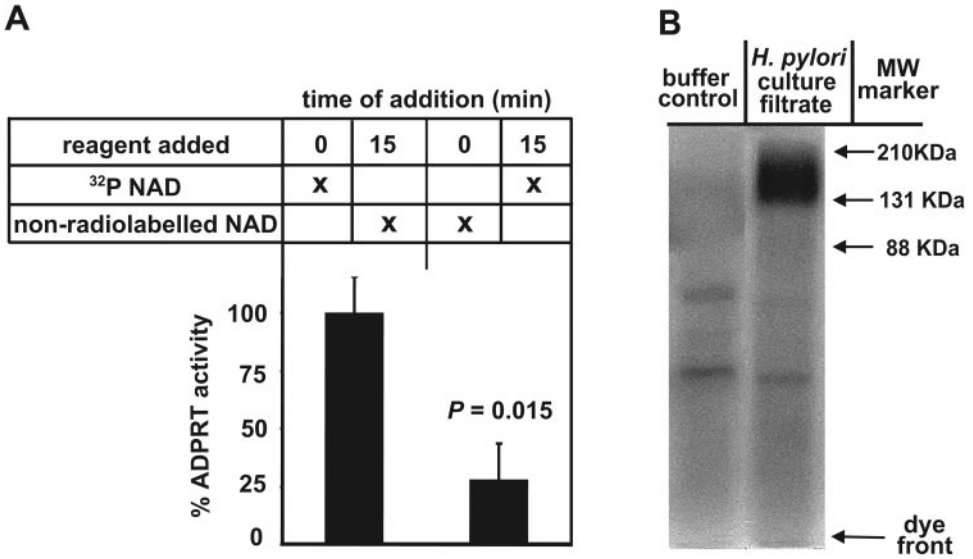

FIG. 3. Characterization of the ^{32}P radiolabel acceptor from HeLa cell extracts. The soluble fraction of HeLa cell lysates (1 mg protein/ml) was incubated with *H. pylori* culture filtrates at 25°C. (A) Reactions were conducted in the presence (×) of 50 μM of either [^{32}P-adenylate]NAD or nonradiolabeled NAD. After 15 min, [^{32}P-adenylate]NAD was added to reactions that were initiated with nonradiolabeled NAD, while nonradiolabeled NAD was added to reactions that were initiated with [^{32}P-adenylate]NAD for a final concentration in each case of 50 μM NAD. The extent of ^{32}P radiolabel transfer after 30 min was scored by scintillation counting of the TCA-precipitated material within the reaction mixture. Each reaction was performed in triplicate, with the experiment performed at least three separate times. Error bars indicate standard deviations, and the *P* values were calculated from the Student *t* test relative to the reaction mixture containing both *H. pylori* culture filtrates and the soluble fraction of HeLa cell lysates. A *P* value of <0.05 was considered significant. (B) Reactions were conducted for 15 min in the presence of 50 μM of [^{32}P-adenylate]NAD. After 15 min, the samples were fractionated by SDS-PAGE (12%), the gel was dried, and the results were evaluated after phosphorimaging the dried gel.

less ($P = 0.015$) in reactions incubated initially with nonradiolabeled NAD versus those incubated initially with radiolabeled DNA (Fig. 3A). Our results are consistent with the idea that the ^{32}P-acceptor in HeLa cell lysates is limiting and suggest that the transfer of ^{32}P radiolabel may be to a specific eukaryotic acceptor.

To further test the idea that a component within *H. pylori* culture filtrate mediates the transfer of ^{32}P radiolabel to a specific eukaryotic acceptor, *H. pylori* culture filtrate (1 mg protein/ml) was incubated with freshly prepared HeLa cell extract (1 mg protein/ml) or phosphate-buffered saline (pH 7.2) in the presence of 50 μM [^{32}P-adenylate]NAD at 25°C. After 15 min, the reaction mixtures were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were dried and then evaluated for the presence of labeled factors after phosphorimaging. These experiments revealed a dominant radiolabeled band that migrated between the 131- and 210-kDa molecular mass markers (Fig. 3B). Although several other radiolabeled species were detected at lower molecular masses, it was estimated by pixel analysis that the high-molecular-mass species accounted for >95% of the incorporated radiolabel. Even when employing lower percentage SDS-PAGE gels or using shorter phosphorimaging times, we never detected more than a single high-molecular-mass, radiolabeled band (data not shown). Collectively, these results support the notion that the *H. pylori*-mediated transfer of ^{32}P radiolabel may be to a specific eukaryotic acceptor. Based on the high molecular mass of the dominant radiolabeled band, it is likely that the mammalian ADP-ribose acceptor does not correspond to previously identified eukaryotic targets of other bacterial ADP-ribosyltransferases, which include elongation factor-2, $G_s\alpha$, $G_i\alpha$, small GTP-binding proteins (Ras and Rho), and actin monomers, all of which have predicted molecular masses of less than 131 kDa.

Notably, the transfer of ^{32}P radiolabel to a high-molecular-mass species is not idiosyncratic to culture filtrates prepared from *H. pylori* 26695. Culture filtrates prepared from two commonly used strains, J99 (ATCC no. 700824) and 60190 (ATCC no. 49503), also stimulated the ^{32}P radiolabel transfer into TCA-precipitated material (data not shown), indicating that the activity is not specific to strain 26695. Moreover, substitution for the HeLa extracts with the soluble fraction of extracts prepared from Chinese hamster ovary cells (CHO-K1 cells; ATCC no. CCL-61), African green monkey kidney cells (Vero cells; ATCC no. CCL-81), or human gastric adenocarcinoma cells (AGS cells; ATCC no. CRL-1739) all yielded a radiolabeled high-molecular-mass species similar to that found with HeLa cells, but only in the presence of *H. pylori* culture filtrates (data not shown), indicating that the acceptor is not idiosyncratic to HeLa cells.

Evidence that *H. pylori*-dependent transfer of ^{32}P radiolabel in the presence of NAD is ADP ribosylation. To more directly test for the existence of an ADP-ribosyltransferase within *H. pylori* culture filtrates, we used an approach that discriminates the active site of ADP-ribosyltransferases with exquisite specificity. In the absence of an ADP-ribose acceptor, UV radiation stimulates photo-cross-linking between the nicotinamide ring of NAD and a single glutamic acid residue that is universally conserved within the active site of ADP-ribosyltransferases (1, 25). The extent of the photo-cross-linking reaction can be monitored by scoring for the covalent transfer of radiolabeled nicotin-

amide from NAD to TCA-precipitable material (e.g., the ADP-ribosyltransferase) while concurrently scoring for the loss of ADP ribosylation activity due to modification of the active site. This probe has been used to map the conserved active-site glutamic acid residue in diphtheria toxin (8, 9), *Pseudomonas aeruginosa* exotoxin A (16), and pertussis toxin (2).

H. pylori culture filtrate (4 mg protein/ml) was incubated at 0°C in 20 mM Tris (pH 7.6), 10 mM NaCl with either [^{3}H-nicotinamide]NAD or [^{32}P-adenylate]NAD (50 μM) in the absence or presence of UV radiation (positioned 5 cm under a germicidal 254-nm UV light source [30W Philips]). The extent of radiolabel transferred at predetermined time points was scored by scintillation counting of the TCA-precipitated material within the reaction mixture. These experiments revealed that the amount of ^{3}H radiolabel transferred to TCA-precipitated material increased with time and was significantly higher in the presence of UV radiation (Fig. 4A). In contrast, the amount of ^{32}P radiolabel detected within the TCA-precipitated fraction did not increase over time in either the presence or the absence of UV radiation. Data from these experiments indicate that UV radiation stimulates the transfer of nicotinamide to a component within *H. pylori* culture filtrate. In order to visualize the radiolabeled *H. pylori* factor, these experiments were repeated except for substituting [^{14}C-nicotinamide]NAD for [^{3}H-nicotinamide]NAD. At the completion of each reaction, the sample was fractionated by SDS-PAGE, and the gels were visualized by phosphorimaging. Despite extensive exposure times, a distinct radiolabeled band could not be readily visualized within the gels (data not shown), which suggests that the amount of ^{14}C-nicotinamide-labeled *H. pylori* factor within our culture filtrate preparations was below the detection threshold.

To test the effects of the UV cross-linking reaction on the putative *H. pylori* ADP-ribosyltransferase activity, *H. pylori* culture filtrate (1 mg protein/ml) was incubated at 0°C in 20 mM Tris (pH 7.6), 10 mM NaCl with [^{3}H-nicotinamide]NAD (200 μM) in the absence or presence of UV radiation. At predetermined time points, aliquots were withdrawn and tested for ^{32}P radiolabel transfer activity by incubating for 15 min with HeLa cell lysates (1 mg/ml) in the presence of [^{32}P-adenylate]NAD (50 μM), 20 mM Tris (pH 7.6), 10 mM NaCl at 25°C. The extent of radiolabel transferred at predetermined time points was scored by scintillation counting (within the ^{32}P channel) of the TCA-precipitated ^{32}P radiolabel within the reaction mixture. These experiments revealed that preincubation of *H. pylori* culture filtrate with both NAD and UV radiation resulted in a time-dependent loss of ^{32}P radiolabel transfer activity (Fig. 4B). In contrast, there was essentially no loss of activity when culture filtrates were incubated in the absence of either NAD or UV radiation (Fig. 4B). These results strongly support the presence of an ADP-ribosyltransferase activity within *H. pylori* culture filtrates.

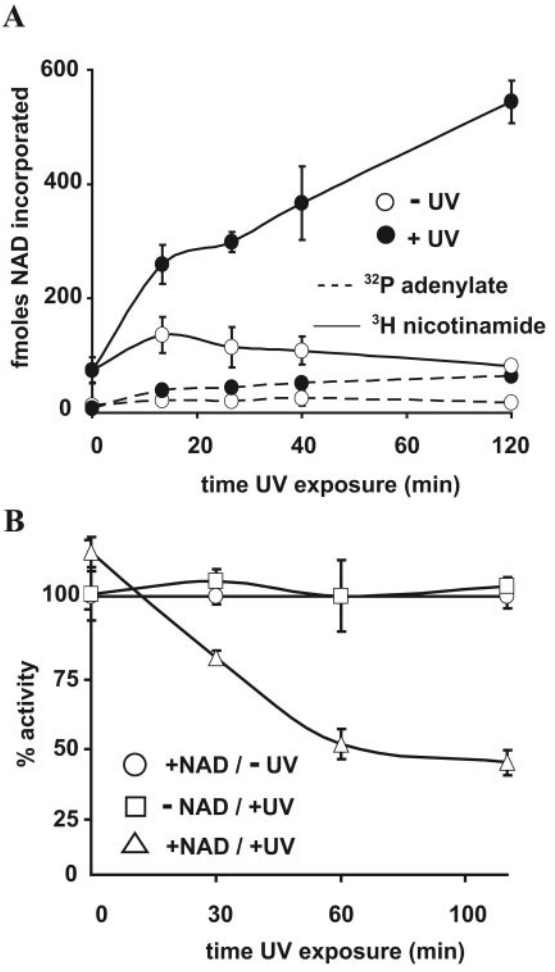

FIG. 4. The *H. pylori* factor mediating ADP ribosylation of the HeLa target protein is susceptible to UV photolabeling with NAD. *H. pylori* 26695 culture filtrate (4 mg protein/ml) was incubated at 0°C with either [^{3}H-nicotinamide]NAD (A and B) or [^{32}P-adenylate]NAD (panel A only) (50 μM [A] or 200 μM [B]) in the presence or absence of UV radiation (as generated by a germicidal 254-nm UV light source [30W Philips]). (A) The extent of radiolabel transferred at predetermined time points was scored by scintillation counting of the TCA-precipitated material within the reaction mixture. The data are displayed as the amount of NAD incorporated as a function of time when incubated with either [^{3}H-nicotinamide]NAD or [^{32}P-adenylate]NAD in the presence or absence of UV radiation. (B) At predetermined times, aliquots were withdrawn and incubated an additional 15 min with HeLa cell lysates (1 mg protein/ml) in the presence of [^{32}P-adenylate]NAD (50 μM), 20 mM Tris (pH 7.6), 10 mM NaCl at 25°C. The extent of ^{32}P radiolabel transferred at predetermined time points was scored by scintillation counting of the TCA-precipitated ^{32}P radiolabel within the reaction mixture. The data are presented as the activity relative to aliquots withdrawn from a control incubated at 0°C in the absence of NAD and UV radiation. Each reaction was performed in triplicate, with each experiment performed at least three separate times. Error bars indicate standard deviations.

Conclusions. Collectively, our data support the presence of an ADP-ribosyltransferase activity within the culture filtrates of several strains of *H. pylori* that selectively modifies a soluble target protein found within several mammalian cell lines from different origins (e.g., human, nonhuman primate, or nonprimate). Based on these results, we are currently working to further characterize this activity, identify the *H. pylori*-derived factor that is responsible for the putative ADP ribosylation activity, and identify the mammalian ADP-ribose acceptor. Interestingly, PSI BLAST analysis of the *H. pylori* genome versus all known bacterial ADP-ribosyltransferases did not reveal an obvious candidate ADP-ribosyltransferase based on homology to conserved active site features (13). The strong association of bacterial-derived ADP-ribosyltransferase activ-

ity with a number of important bacterial toxins as well as a number of effectors secreted by pathogenic bacteria into mammalian cells (1) suggests the intriguing possibility that *H. pylori* might also generate a factor that contributes to pathogenesis of the bacterium by modifying a host target protein through ADP ribosylation.

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