

Received 17 July; accepted 24 October 1991.

1. Santen, R. J. in *Endocrinology and Metabolism* (eds Felig, P., Baxter, J. D., Broadus, A. E. & Frohman, L. A.) 821-905 (McGraw-Hill, New York, 1987).
2. Ewing, L. L. & Robaire, B. *Ann. N.Y. Acad. Sci.* **564**, 1-302 (1989).
3. Foulkes, N. S., Borrelli, E. & Sassone-Corsi, P. *Cell* **64**, 739-749 (1991).
4. Hoefler, J. P., Meyer, T. E., Yun, Y., Jameson, J. L. & Habener, J. F. *Science* **242**, 1430-1433 (1988).
5. Gonzalez, G. A. et al. *Nature* **337**, 749-752 (1989).
6. Foulkes, N. S., Laoidé, B. M., Schlotter, F. & Sassone-Corsi, P. *Proc. natn. Acad. Sci. U.S.A.* **88**, 5448-5452 (1991).
7. Mellon, P. L., Clegg, C. H., Correll, L. A. & McKnight, G. S. *Proc. natn. Acad. Sci. U.S.A.* **86**, 4887-4891 (1989).
8. Gonzalez, G. A., Menzel, P., Leonard, J., Fischer, W. & Montminy, M. R. *Molec. cell. Biol.* **11**, 1306-1312 (1991).
9. Courcy, A. J. & Tjian, R. *Cell* **55**, 887-898 (1989).
10. Lee, C. Q., Yun, Y., Hoefler, J. P. & Habener, J. F. *EMBO J.* **9**, 4455-4465 (1990).
11. Willison, K. & Ashworth, A. *Trends Genet.* **3**, 351-355 (1987).
12. Dym, M. in *Histology Cell and Tissue Biology* (ed. Weiss, L.) 1000-1053 (Elsevier Biomedical, New York, 1983).
13. Lyon, M. F. & Hawkes, S. G. *Nature* **227**, 1217-1219 (1970).
14. Moutier, R. in *The Laboratory Animal in the Study of Reproduction* (eds Antikatzides, T., Erichsen, S. & Spiegel, A.) 5-7 (Fischer, Stuttgart, 1976).
15. Beebe, S. J. et al. *Molec. Endocrin.* **4**, 465-475 (1990).
16. McKnight, G. S. et al. *Recent Prog. Horm. Res.* **44**, 307-335 (1988).
17. Auffray, C. & Rougeon, F. *Eur. J. Biochem.* **107**, 303-314 (1980).
18. Sassone-Corsi, P., Ransone, L. J., Lamph, W. W. & Verma, I. M. *Nature* **336**, 692-694 (1988).
19. Mather, J. P. *Biol. Reprod.* **23**, 243-251 (1980).
20. Kilpatrick, D. L., Borland, K. & Jin, D. F. *Proc. natn. Acad. Sci. U.S.A.* **84**, 5695-5699 (1987).

ACKNOWLEDGEMENTS. We thank E. Borrelli, J. R. Naranjo, B. Laoidé, D. Masquillier and J. P. Montmayeur for discussions, C. Passananti, M. Russo and P. Amati (University of Rome) for RNA samples from fractionated testis cells, Ph. Avner (Institut Pasteur) for the Tfm/Y mice, S. G. McKnight (Washington University) for pCαEV, J. Auwerx, P. Pevet, B. Laoidé, D. Masquillier, M. Acker and J. P. Montmayeur for materials, F. Schlotter for technical assistance and B. Boulay and F. Haenel for help in the preparation of the manuscript. B.M. was on leave from the Cajal Institute for Neuroscience, Madrid, N.S.F. was supported by a long-term EMBO fellowship. This work was supported by the CNRS, INSERM, Association pour la Recherche contre le Cancer and Rhone-Poulenc-Rorer.

Mapping of the protein import machinery in the mitochondrial outer membrane by crosslinking of translocation intermediates

Thomas Söllner, Joachim Rassow, Martin Wiedmann*†, Jens Schlossmann, Petra Keil, Walter Neupert & Nikolaus Pfanner‡

Institut für Physiologische Chemie, Universität München, Goethestrasse 33, W-8000 München 2, Germany
* Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften, O-1115 Berlin-Buch, Germany

MITOCHONDRIA contain a complex machinery for the import of nuclear-encoded proteins^{1,2}. Receptor proteins exposed on the outer membrane surface are required for the specific binding of precursor proteins to mitochondria, either by binding of cytosolic signal recognition factors or by direct recognition of the precursor polypeptides¹⁻⁵. Subsequently, the precursors are inserted into the outer membrane at the general insertion site GIP (general insertion protein)⁶⁻¹⁰. Here we report the analysis of receptors and GIP by crosslinking of translocation intermediates and by coimmunoprecipitation. Surface-accumulated precursors were crosslinked to the receptors MOM19 and MOM72, suggesting a direct interaction of preproteins with surface receptors. We identified three novel mitochondrial outer membrane proteins, MOM7, MOM8, and MOM30 that, together with the previously identified MOM38, seem to form the GIP site and are present in the mitochondrial receptor complex.

The precursor of the ADP/ATP carrier, the most abundant protein of the mitochondrial inner membrane, was synthesized in rabbit reticulocyte lysate in the presence of ³⁵S-methionine¹¹. To accumulate the precursor at the mitochondrial outer mem-

brane (receptor stage and GIP stage), isolated *Neurospora crassa* mitochondria were incubated with the precursor in the presence of ATP, but in the absence of a membrane potential across the inner membrane¹¹⁻¹³. The mitochondria were incubated with the homobifunctional crosslinking reagent DSS (disuccinimidyl suberate) or the heterobifunctional crosslinking reagent sulpho-MBS (*m*-maleimidobenzoyl-*N*-hydroxysulphosuccinimide ester), followed by SDS-PAGE and fluorography. Figure 1a shows the crosslink products and their assignment to components of the import machinery as outlined below (the products containing MOM72 have slightly different apparent sizes depending on the crosslinker used; significant crosslinking to MOM30 was only observed with sulpho-MBS, and to MOM19 with DSS). When the mitochondria were pretreated with a low concentration of protease (leading to a degradation of surface proteins, but not of the membranes) only nonproductive unspecific binding of the precursor was possible^{4,7,11,14} and none of the crosslink products was generated (not shown). As MOM7, MOM8 and MOM30 are not degraded under these conditions (see below), the formation of these crosslink products must depend on the specific association of ADP/ATP carrier with mitochondria.

When isolated mitochondria and precursor were incubated at low levels of ATP and in the absence of a membrane potential, the precursor only bound to the mitochondrial surface and the insertion into the GIP site was inhibited. The exposure of this precursor on the mitochondrial surface was evidenced by its accessibility to proteases and to antibodies directed against the ADP/ATP carrier^{4,7,11-13}. The precursor was on the correct import pathway, as it followed the further import steps on addition of ATP and generation of a membrane potential^{4,7,11,12}. With this surface-accumulated precursor, only the crosslink products to MOM19 and MOM72 were formed (Fig. 1b, d). These products were identified by immunoprecipitation with specific antibodies. The apparent relative molecular masses of about 50,000 (*M_r* 50 K) and 100 K of the crosslink products fit to crosslinks between one molecule of ADP/ATP carrier (30K) and one molecule of MOM19 (19 K) or MOM72 (72 K), respectively. The crosslink product of 170 K probably consists of ADP/ATP carrier and a homodimer of MOM72, as a fraction of MOM72 is present as a noncovalent homodimer which is stable enough to survive the heating in SDS-containing sample buffer (Fig. 2; T. S. and N. P., unpublished data). As controls, none of the crosslink products was precipitated with preimmune antibodies or antibodies against porin, the most abundant outer membrane protein, whereas all were recognized by antibodies directed against ADP/ATP carrier as expected (Fig. 1b). The selective crosslinking of ADP/ATP carrier arrested at the mitochondrial surface to MOM19 and MOM72 agrees with our previous finding that both proteins are involved in the import of ADP/ATP carrier⁵ and provides strong evidence that these import receptors function by direct interaction with the precursor polypeptide. The receptor-mediated import of a chemically pure preprotein into purified mitochondria in the absence of cytosolic cofactors¹⁵ supports this conclusion.

The crosslink products with MOM7, MOM8 and MOM30 were not generated to an appreciable extent with the precursor accumulated at the receptor stage (Fig. 1b, d), suggesting that those crosslink products may be related to the GIP stage. To test this directly we made use of the observation that the precursors accumulated at the GIP site were protected against externally added protease, whereas the receptor-accumulated precursors (and the receptors themselves) were degraded by low concentrations of protease^{6,7,11,12}. ADP/ATP carrier was accumulated at the outer membrane, the mitochondria were treated with protease and then subjected to crosslinking. As expected, the crosslink products with MOM72 or MOM19 were not formed after a treatment with low protease concentrations. By contrast, the crosslink products with MOM7, MOM8 and MOM30 could still be generated (Fig. 1c, d), demonstrating that these three

† Present address: Rockefeller Research Laboratory, Sloan-Kettering Institute, New York, New York 10021, USA.

‡ To whom correspondence should be addressed.

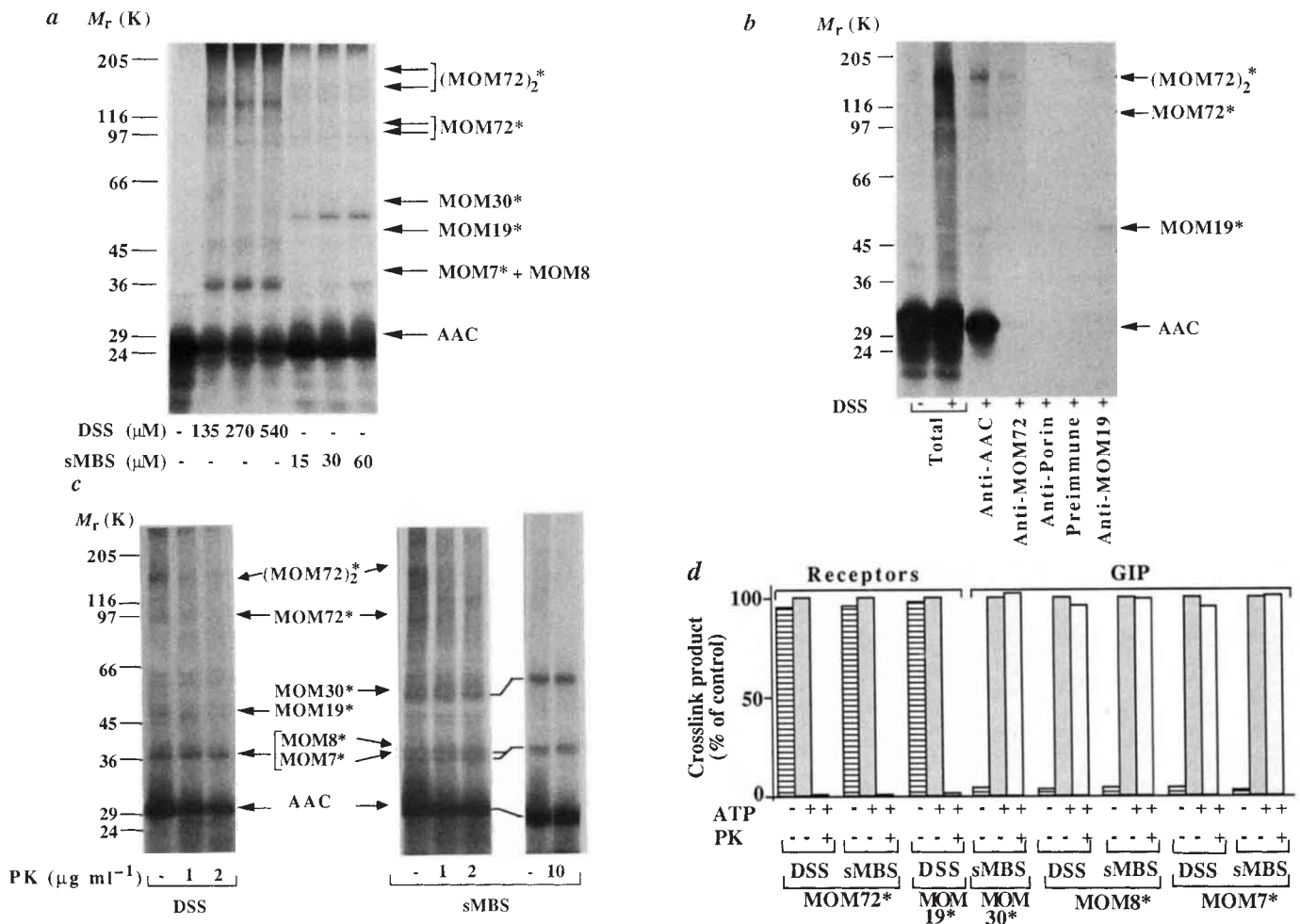


FIG. 1 Identification of mitochondrial outer membrane proteins close to the precursor of the ADP/ATP carrier. *a*, Crosslink products of the ADP/ATP carrier accumulated at the outer membrane in the absence of a membrane potential (receptor stage + GIP stage). *b*, Identification of MOM19 and MOM72 in the crosslink products on the mitochondrial surface by immunoprecipitation. *c*, Identification of MOM7, MOM8 and MOM30 in the crosslink products at the GIP stage (+PK). *d*, Stage-specific formation of crosslink products at the mitochondrial surface (-ATP) or at the GIP stage (+PK). AAC, ADP/ATP carrier; (MOM72)₂, homodimer of MOM72; PK, proteinase K; *, products between AAC and mitochondrial outer membrane proteins. METHODS. *a*, Rabbit reticulocyte lysate (4 μ l) containing ³⁵S-labelled precursor of AAC was incubated with isolated *N. crassa* mitochondria (25 μ g mitochondrial protein) in the absence of a membrane potential for 20 min at 25 °C as described^{4,6,10,11}. The mitochondria was reisolated by centrifugation through a 500 mM sucrose cushion and washed in SEM-buffer (250 mM sucrose, 1 mM EDTA, 10 mM MOPS, pH 7.2). Crosslinking was done in 1 mM SEM with DSS (Pierce) or sMBS (Pierce) for 30 min at 0 °C. After addition of 100 mM tris(hydroxymethyl)aminomethane (pH 7.4) or 100 mM glycine (pH 7.2) plus 0.1% (v/v) β -mercaptoethanol, respectively, and incubation for 30 min at 0 °C, the proteins were analysed by SDS-PAGE and fluorography¹¹. *b*, Isolated mitochondria and reticulocyte lysate containing precursor of AAC

were pretreated with apyrase (5 U ml⁻¹) as described^{4,11,12}, incubated for 7 min at 25 °C and further treated as described above. The samples were dissolved in buffer containing 2% SDS. The first two samples ('total') were directly subjected to SDS-PAGE, whereas the other samples were diluted 20-fold with buffer containing 1% Triton X-100 and 300 mM NaCl and immunoprecipitations with the indicated antibodies were done^{3,10,11}. Minor bands of noncrosslinked precursor were occasionally found in some precipitates independently of the type of antiserum used, apparently representing unspecific precipitation of a very minor fraction of the large amount of labelled precursor. *c*, The experiment was done as described for *a* with the following modifications. After accumulation of AAC at the outer membrane, treatment with proteinase K was as indicated^{6,11}. For the crosslinking, DSS (200 μ g ml⁻¹) and sMBS (25 μ g ml⁻¹) were used. *d*, The experiment was done as in *b* and *c* (10 μ g ml⁻¹ proteinase K). The crosslink products were quantified by laser densitometry using a calibration curve¹¹. The amount of each crosslink product obtained with AAC accumulated at receptor plus GIP stages was set to 100% (middle column of each set) (MOM72* includes the homodimer). The crosslink products with MOM7 and MOM8 were extracted (>95%) from the membranes at pH 11.5 (100 mM Na₂CO₃)^{3,4}, whereas the product containing MOM30 was carbonate-resistant (>95% in the pellet).

components are close to the GIP site. The highest efficiency of crosslinking was observed for the products with MOM7 and MOM8 (up to 15% of the accumulated ADP/ATP carrier). ADP/ATP carrier accumulated at the GIP site was extracted from the membranes at pH 11.5, suggesting that it was embedded in a hydrophilic environment⁶. The crosslink product with MOM30 was not extracted at pH 11.5, indicating that MOM30 is anchored in the outer membrane. The crosslink products with MOM7 and MOM8 were extractable at pH 11.5 (see legend to Fig. 1), suggesting that these proteins are not integral membrane proteins; they are probably associated with the outer membrane through interaction with other pro-

teins. The 38 K protein MOM38 has previously been identified as part of GIP (ref. 10). A crosslink product between ADP/ATP carrier at the GIP-stage and *N. crassa* MOM38 represented only a minor band, whereas a more efficient crosslinking was found with *Saccharomyces cerevisiae* mitochondria (T. S. and N. P., unpublished data). The considerable difference in the efficiency of crosslinking of precursors to MOM38 (=ISP42) was also observed previously^{8,16} and is obviously due to the tightly folded structure of native MOM38 (ref. 10).

Thus, at least four proteins, MOM7, MOM8, MOM30 and MOM38, seem to be close to a precursor arrested at the GIP site. On the other hand, the purified mitochondrial receptor

complex that contained ADP/ATP carrier bound to GIP consisted of the four proteins MOM19, MOM22 (a surface protein of unknown function¹⁰), MOM38 and MOM72. MOM19, MOM22 and MOM72 are protease-accessible in intact mitochondria, leaving MOM38 as the only candidate for the GIP site¹⁰. How can this apparent controversy be resolved? Because the mitochondrial receptor complex is a very labile complex that can be partially or completely dissociated under relatively mild conditions¹⁰, some components of the complex may have been lost by the purification procedure used (whereas the interaction of MOM38 with precursors arrested at the GIP stage apparently is stable enough to keep these precursors associated with the complex during the purification). When glycerol was included in the isolation buffer, the complex purified with an affinity matrix carrying anti-MOM19 antibodies contained three proteins of 7K, 8K and 30K in addition to the four known components (Fig. 2). With highly purified mitochondrial outer membranes (no detectable amounts of inner membranes), about 25 different polypeptides are resolved by SDS-PAGE^{3,4}. As expected, the three new proteins found in the receptor complex of the outer membrane represent previously unassigned outer membrane proteins (Fig. 2). Under the various conditions that

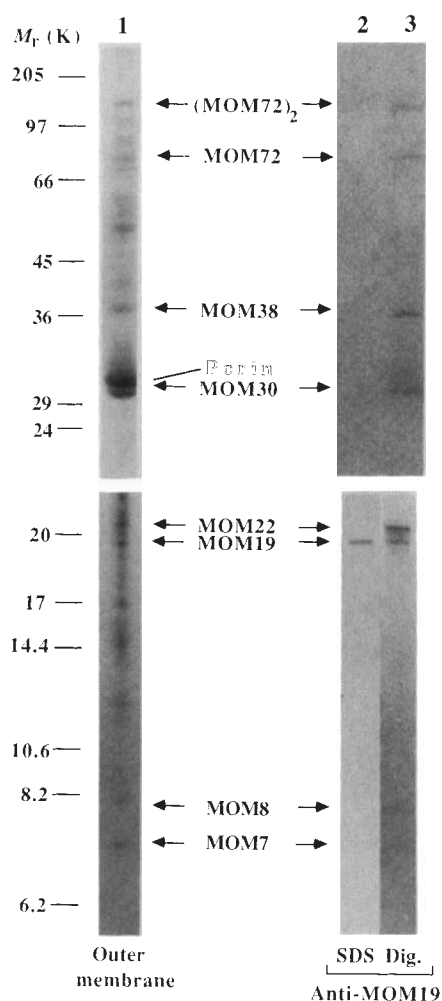


FIG. 2 Identification of three novel proteins, MOM7, MOM8 and MOM30, in the mitochondrial receptor complex. Lane 1, purified mitochondrial outer membrane^{3,4}. Lane 2, reactivity of anti-MOM19 antibodies with mitochondrial proteins under stringent conditions (denaturation in SDS followed by dilution in Triton X-100 buffer^{3,10}). Lane 3, purified receptor complex (Dig., digitonin-containing buffer).

METHODS. The purification of the mitochondrial receptor complex was done as described¹⁰ except that 10% (v/v) glycerol was included in the digitonin-containing buffer and the anti-MOM19 antibodies were covalently coupled to CNBr-activated Sepharose 4B (Pharmacia).

led to the identification of the three new components, no proteolytic degradation of proteins was observed. Proteins that are very sensitive to various proteases, in particular MOM72 and loosely folded precursor proteins, were fully stable at these conditions^{3,4,6,10}, excluding that the new components are proteolytic breakdown products.

We have thus identified three new proteins located in the outer membrane by the following methods: (1) crosslinking to ADP/ATP carrier arrested at the GIP site; (2) an improved procedure for the purification of the receptor complex of the outer membrane; and (3) comparison of the apparent sizes of the proteins with that of the components of purified outer membranes. The ADP/ATP carrier arrested at the GIP site of the outer membrane is exclusively accumulated at this stage, because a dissipation of the membrane potential completely blocks its further transport into the inner membrane and the treatment with protease degrades all precursor molecules at the receptor stage^{4,6,7,11,12}. As the purified receptor complex contains the GIP site¹⁰, the three new components identified by crosslinking are most likely to represent the three new proteins found in the receptor complex. With MOM7 and MOM8 this is also evident from their apparent size as all other outer membrane proteins resolved have apparent sizes of at least 12K. MOM7, MOM8, MOM19, MOM22, MOM30, MOM38 and MOM72 are present in the mitochondrial receptor complex in molar ratios of about 1:1:1:0.6:0.4:1:0.5 (± 0.15), respectively. Mitochondrial proteins of about 30K (28K–32K) were identified by their affinity to mitochondrial presequences^{17–20}. The relation of the 30K proteins to each other and to MOM30 is unknown.

Our study leads to the following key conclusions on the functioning of the protein import apparatus in the mitochondrial outer membrane (Fig. 3). (1) MOM19 and MOM72 act as direct receptors for the precursor polypeptides, representing the first stage of binding of precursors to mitochondria. (2) Precursor proteins are inserted into the outer membrane at the general insertion site GIP that seems to be formed by (at least) four proteins, MOM7, MOM8, MOM30 and MOM38. The relatively

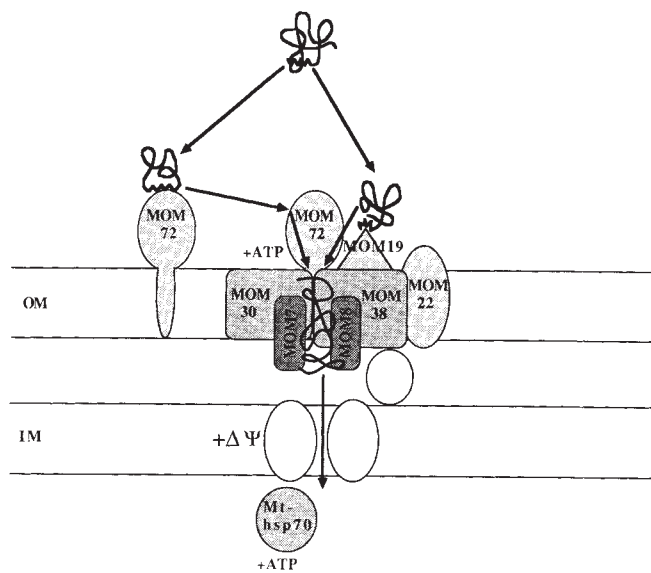


FIG. 3 Hypothetical model of the mitochondrial machinery for recognition and membrane insertion of precursor proteins. The receptor complex in the outer membrane (OM) consists of the two preprotein receptors MOM72 and MOM19, four proteins (MOM38, MOM30, MOM8 and MOM7) close to the general insertion site GIP, and MOM22 (with unknown function). The binding of preproteins to MOM72 seems to occur outside the complex, and MOM72 subsequently associates with the complex¹⁰, indicating a dynamic nature of the receptor complex. Preproteins accumulated at the GIP site are exposed to the intermembrane space²¹. Translocation into or across the inner membrane (IM) requires a membrane potential $\Delta\Psi$ (ref. 22) and the heat shock protein hsp70 in the matrix^{16,23,24}. MOMy, mitochondrial outer membrane protein with an apparent size of yK.

high efficiency of crosslinking of GIP intermediates to MOM7 and MOM8 would fit to a participation of these proteins in the formation of an insertion pore in the outer membrane. (3) The receptors and GIP are assembled in a high M_r complex of the outer membrane that consists of (at least) seven proteins. □

Received 24 June; accepted 12 November 1991.

1. Baker, K. P. & Schatz, G. *Nature* **349**, 205–208 (1991).
2. Pfanner, N., Söllner, T. & Neupert, W. *Trends Biochem. Sci.* **16**, 63–67 (1991).
3. Söllner, T., Griffiths, G., Pfaller, R., Pfanner, N. & Neupert, W. *Cell* **59**, 1061–1070 (1989).
4. Söllner, T., Pfaller, R., Griffiths, G., Pfanner, N. & Neupert, W. *Cell* **62**, 107–115 (1990).
5. Steger, H. F. *et al.* *J. Cell Biol.* **111**, 2353–2363 (1990).
6. Pfanner, N. & Neupert, W. *J. Biol. Chem.* **262**, 7528–7536 (1987).
7. Pfaller, R., Steger, H. F., Rassow, J., Pfanner, N. & Neupert, W. *J. Cell Biol.* **107**, 2483–2490 (1988).
8. Vestweber, D., Brunner, J., Baker, A. & Schatz, G. *Nature* **341**, 205–209 (1989).
9. Baker, K. P., Schaniel, A., Vestweber, D. & Schatz, G. *Nature* **348**, 605–609 (1990).
10. Kiebler, M. *et al.* *Nature* **348**, 610–616 (1990).
11. Söllner, T., Rassow, J. & Pfanner, N. *Meth. Cell Biol.* **34**, 345–358 (1991).
12. Pfanner, N., Tropsch, M. & Neupert, W. *Cell* **49**, 815–823 (1987).
13. Söllner, T., Pfanner, N. & Neupert, W. *FEBS Lett.* **229**, 25–29 (1988).
14. Pfaller, R., Pfanner, N. & Neupert, W. *J. Biol. Chem.* **264**, 34–39 (1989).
15. Becker, K., Guiard, B., Rassow, J., Söllner, T. & Pfanner, N. *J. Biol. Chem.* **267**, (in the press).
16. Scherer, P. E., Krieg, U. C., Hwang, S. T., Vestweber, D. & Schatz, G. *EMBO J.* **9**, 4315–4322 (1990).
17. Gillespie, L. L. *J. Biol. Chem.* **262**, 7939–7942 (1987).
18. Ono, H. & Tuboi, S. *J. Biochem.* **107**, 840–845 (1990).
19. Pain, D., Murakami, H. & Blobel, G. *Nature* **347**, 444–449 (1990).
20. Font, B. *et al.* *FEBS Lett.* **279**, 105–109 (1991).
21. Rassow, J. & Pfanner, N. *FEBS Lett.* (in the press).
22. Martin, J., Mahlke, K. & Pfanner, N. *J. Biol. Chem.* **266**, 18051–18057 (1991).
23. Kang, P. J. *et al.* *Nature* **348**, 137–143 (1990).
24. Ostermann, J. *et al.* *FEBS Lett.* **277**, 281–284 (1990).

ACKNOWLEDGEMENTS. We thank T. Rapoport and D. Görlich (Berlin-Buch) for discussions and U. Hanemann and A. Weinzierl for technical assistance. This study was supported by the Sonderforschungsbereich 184.

Allosteric underwinding of DNA is a critical step in positive control of transcription by Hg–MerR

Aseem Z. Ansari*, Mark L. Chael*
& Thomas V. O'Halloran*†‡

* Department of Biochemistry, Molecular Biology and Cell Biology and

† Department of Chemistry, 2145 Sheridan Road,

Northwestern University, Evanston, Illinois 60208–3113, USA

POSITIVE control of transcription often involves stimulatory protein–protein interactions between regulatory factors and RNA polymerase¹. Critical steps in the activation process itself are seldom ascribed to protein–DNA distortions. Activator-induced DNA bending is typically assigned a role in binding-site recognition², alterations in DNA loop structures³ or optimal positioning of the activator for interaction with polymerase⁴. Here we present a transcriptional activation mechanism that does not require a signal-induced DNA bend but rather a receptor-induced untwisting of duplex DNA. The allosterically modulated transcription factor MerR is a repressor and an Hg(II)-responsive activator of bacterial mercury-resistance genes^{5–7}. *Escherichia coli* RNA polymerase binds to the MerR–promoter complex but cannot proceed to a transcriptionally active open complex until Hg(II) binds to MerR (ref. 6). Chemical nuclease studies show that the activator form, but not the repressor, induces a unique alteration of the helical structure localized at the centre of the DNA-binding site⁶. Data presented here indicate that this Hg–MerR-induced DNA distortion corresponds to a local underwinding of the spacer region of the promoter by about 33° relative to the MerR–operator complex. The magnitude and the direction of the Hg–MerR-induced change in twist angle are consistent with a positive control mechanism involving reorientation of conserved, but suboptimally phased, promoter elements and are consistent with a role for torsional stress in formation of an open complex.

‡ To whom correspondence should be addressed.

The *mer* operator is located directly between the –10 and –35 hexameric RNA polymerase-binding elements of the P_T promoter. In both the repressor and activator states, MerR binds to a site centred 26 base pairs (bp) upstream from the transcription start site and in the region footprinted by *E. coli* σ^{70} RNA polymerase (RNAP)^{7,8}. This spacer DNA between the –10 and –35 hexamers of the P_T promoter is 19 bp, two bp longer and about 70° out of phase relative to a consensus *E. coli* promoter⁹. Genetic studies of deletions in the spacer region by Brown and coworkers have demonstrated the importance of a 19-bp spacer: deletion of one or two base pairs results in a stronger promoter that is no longer dependent on Hg–MerR for activation^{10,11}. Footprinting results on open complexes *in vivo* are consistent with a distortion at the centre of the operator¹².

The role of protein-induced DNA bending in this allosterically modulated DNA distortion was probed using an electrophoretic mobility shift assay^{13,14}. Analysis of the mobility of 151-bp restriction fragments with a *mer* operator at various positions (Fig. 1a) indicates that MerR induces a small bend in the operator site of 25° ± 10° (Fig. 1b, lanes 6–10). This bend does not change on binding of the coeffector Hg(II) (Fig. 1b, lanes 11–15). Similar results were obtained with a circularly permuted 201-bp wild-type operator/promoter fragment.

Because a change in bending did not correlate with the repressor-to-activator transition, an alternative method for

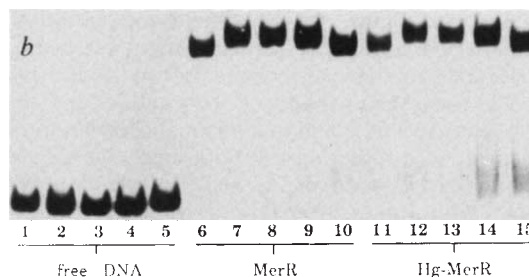
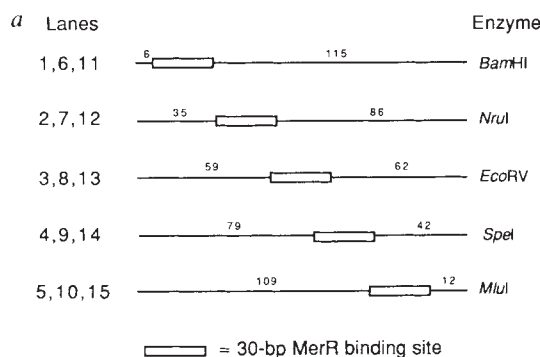


FIG. 1 Determination of MerR and Hg–MerR-induced DNA bending by circular permutation. *a*, Five 151-bp restriction fragments used in *b* indicating the relative position of the MerR-binding site in relation to the ends of the fragments. *b*, The mobility of MerR–DNA complexes using the DNA fragments listed in *a*. Gel mobility shift assays were done with 5 pM DNA (lanes 1–15), 50 nM MerR (lanes 6–15), and 0.5 μ M Hg(II) (lanes 11–15).

METHODS. The plasmid pMB10 was made by inserting the 30-base oligonucleotide duplex: 5'-GCTTGACTCCGATACATGAGTACCGAAGTAA-3' into the unique *Xba*I restriction site of the plasmid pBend2 (ref. 14) after end-filling the *Xba*I site with the Klenow fragment of DNA pol. In all, 11 151-bp restriction fragments were isolated, end-labelled with [γ -³²P]ATP and T4 polynucleotide kinase and analysed by gel mobility shift assays^{13,14}. Gel shifts were carried out in 20 μ l final volume of 10 mM Tris-HCl (pH 8), 60 mM KCl, 0.1 mM EDTA (pH 8), 5 μ g ml⁻¹ BSA, 1 mM DTT and 5% glycerol. After incubation at 23 °C for 45 min, 2 μ l 50% glycerol was added and the samples were loaded onto a 10% (75:1 acrylamide:bisacrylamide), 1 × TBE gel. Electrophoresis was for 3–5 h at 183 V. The bending locus was graphically determined using the method of Wu and Crothers¹³. The bending angle was calculated using the equation of Thompson and Landy²⁸.