

Hoppe-Seyler's Zeitschrift für Physiologische Chemie

Band 357 – 1. Jahreshälfte

Fortgeführt von A. Kossel, F. Knoop und K. Thomas · Herausgegeben von

A. Butenandt, F. Lynen, G. Weitzel

unter Mitwirkung von K. Bernhard, K. Decker, J. Engel, H. Fritz, B. Hamprecht,

H. Hanson, E. Helmreich, H. Herken, B. Hess, N. Hilschmann, H. Hilz, P. W. Jungblut, P. Karlson,
H. L. Kornberg, K. Kühn, F. Leuthardt, D. Oesterhelt, K. Rajewsky, J. Seelig, G. Siebert, H. Simon,

Hj. Staudinger, W. Stoffel, H. Tuppy, H. Wiegandt, H. G. Wittmann, H. G. Zachau, H. Zahn

Redaktion A. Dillmann, G. Peters



1976

WALTER DE GRUYTER · BERLIN · NEW YORK



© Copyright 1976 by Walter de Gruyter & Co., Berlin.

Alle Rechte, insbesondere die der Übersetzung in fremde Sprachen, vorbehalten. Kein Teil dieser Zeitschrift darf ohne schriftliche Genehmigung des Verlages in irgendeiner Form – durch Photokopie, Mikrofilm oder irgendein anderes Verfahren – reproduziert oder in eine von Maschinen, insbesondere von Datenverarbeitungsmaschinen verwendbare Sprache übertragen oder übersetzt werden. – All rights reserved, including those of translations into foreign languages. No part of this journal may be reproduced in any form – by photoprint, microfilm or any other means – or transmitted or translated into a machine language without written permission from the publisher.

Nach § 54, Abs. 2 URG ist für die photomechanische, xerographische oder in sonstiger Weise bewirkte Anfertigung von Vervielfältigungen der in dieser Zeitschrift erschienenen Beiträge zum eigenen Gebrauch eine Vergütung zu bezahlen, wenn die Vervielfältigung gewerblichen Zwecken dient. Die Vergütung ist nach Maßgabe des zwischen dem Börsenverein des Deutschen Buchhandels e. V., Frankfurt/Main, und dem Bundesverband der Deutschen Industrie in Köln abgeschlossenen Gesamtvertrages von 15. Juli 1970 zu entrichten. Die Weitergabe von Vervielfältigungen, gleichgültig zu welchem Zweck sie hergestellt werden, ist eine Urheberrechtsverletzung und wird strafrechtlich verfolgt. Die hier genannten Vervielfältigungen haben den Vermerk über den Hersteller und die Bezahlung der Lizenzen zu tragen.

Die Wiedergabe von Gebrauchsnamen, Handelsnamen, Warenbezeichnungen und dergleichen in dieser Zeitschrift berechtigt nicht zu der Annahme, daß solche Namen ohne weiteres von jedermann benutzt werden dürfen. Vielmehr handelt es sich häufig um gesetzlich geschützte, eingetragene Warenzeichen, auch wenn sie nicht eigens als solche gekennzeichnet sind. – The quotation of registered names, trade names, trade marks, etc. in this journal does not imply, even in the absence of a specific statement, that such names are exempt from laws and regulations protecting trade marks, etc. and therefore free for general use.

Anzeigenverwaltung: Merkur-Werbung, Dr. K. Jeserich KG, 521 Troisdorf 22, Merkur-Haus, Hauptstraße 23 - 27, Tel. (02241) 42051. Für den Anzeigenteil verantwortlich: Wolfgang Friebe, Troisdorf. – Printed in Germany – Satz: R. & J. Blank, München; Druck: Grafik + Druck, München; Bindearbeiten: F. Steinberger & Sohn, München.

GH 76/488

Autoren-Verzeichnis der ersten Jahreshälfte 1976

- Adamietz, P. 527
 Afting, E.-G. 257, 277
 Agrawal, A. K. 276
 Albert, E. D. 619
 Altenburger, W. 321
 Altendorf, K.-H. 328
 Amzel, L. M. 614
 Anfinzen, C. B. 123
 Ankel, H. 251
 Arndt, R. 247
 Arnold, H. H. 290, 303
 Aschhoff, H. J. 290
 Averdunk, R. 673
 Axel, R. 335

 Back, P. 213, 219
 Baczko, K. 306
 Ball, P. 81
 Bargetzi, J.-P. 867
 Barmetler, A. 248
 Bastian, Ch. 307
 Basu, S. 251
 Bauer, Ch. 248
 Bauer, F.-U. 187
 Bauer, G. 307
 Bauerle, R. 259
 Baur, H. 249
 Baur, R. 308
 Bautz, E. K. F. 318
 Bayev, A. A. 305, 308, 337
 Beato, M. 311
 Beck, G. 493
 Beck, J. P. 493
 Becker, M. L. 277
 Beierstorf, K. 308
 Benecke, B. J. 308
 Beneking, M. 492
 Benes, P. 249
 Berek, C. 616
 Béress, L. 409
 Béress, R. 409
 Berghäuser, J. 249
 Bergheim, E. 249
 Berkhoff, W. 277
 Bermek, E. 721
 Berndt, J. 250, 252
 Berner, W. 250
 Berthold, V. 309
 Besançon, F. 251
 Besemer, J. 334
 Betz, J. 251, 777

 Beyreuther, K. 309
 Binder, C. 751
 Black, S. 616
 Bode, J. 309
 Bodo, G. 121, 125
 Boedtker, H. 315, 325
 Böhmer, R. 310
 Bohlig, H. J. 67
 Bojar, H. 252
 Boll, M. 252
 Bolt, H. M. 351
 Bonnet, J. 293
 Boos, K.-S. 290
 Bose, S. 123
 Bové, J. 250
 Braun, V. 260
 Braun, W. 279
 Bredehorst, R. 51
 Bretzel, G. 153, 253, 487
 Breuer, H. 276, 573
 Breuker, E. 276
 Brodner, O. G. 89
 Brossmer, R. 283
 Brown, C. R. 57
 Brown, D. T. 323
 Brownlee, G. G. 616
 Brunfeldt, K. 713
 Brunner, G. 253
 Bruski, S. 254
 Bucher, D. 103
 Bucher, J. 623
 Buck, F. 302
 Buddecke, E. 272, 285, 641
 Bünemann, H. 310
 Bugany, H. 311
 Buhre, U. 324
 Bujard, H. 308, 311
 Buscher, H.-P. 559

 Calendar, R. 315
 Cardó, M. T. 250
 Čechová, D. 401
 Chen, B. L. 614
 Chen, R. 873
 Cheng, C. C. 616
 Christensen, T. 713
 Chumakov, P. M. 326
 Čihák, A. 345
 Collins, J. 311, 312
 Colman, P. M. 435, 614

 Comer, M. M. 291
 Constantinidis, L. 276
 Corley, L. 123
 Cramer, F. 292, 293, 295, 303
 Cunningham, B. A. 621

 Därr, W. 127
 Dahl, H. 124
 Dahr, W. 254
 Dattagupta, N. 310
 Dauner, H.-O. 147
 Davies, P. J. 341
 Debuch, H. 281, 707, 803
 Decker, K. 263, 427
 Defer, N. 493
 Degré, M. 124
 Deimer, K. H. 447
 Deisenhofer, J. 435, 614
 Dejon, L. 277
 Dew, M. E. 261
 Dieckvoß, G. 287
 Dietl, T. 139, 657
 Dietrich, A. 293
 Dirheimer, G. 293, 298
 Dittgen, R. M. 543
 Doenecke, D. 312
 Doerfler, W. 306, 317, 333, 339
 Dommes, V. 255
 Donner, I. 495, 811
 Dorn, G. 893
 Dose, K. 278
 Drahovsky, D. 313
 Drews, U. 95
 Düllfer, T. 477
 Duntze, W. 255
 Du Pasquier, L. 617
 Durchschlag, G. 256
 Durchschlag, H. 256
 Dutler, H. 299
 Dworzak, E. 259

 Ebel, J. P. 292, 293
 Eckstein, F. 295
 Edelman, G. M. 621
 Edy, V. G. 123
 Egge, H. 288
 Ehrenberg, M. 291
 Ehring, R. 335
 Ehrlich, K. 839

 Eichmann, K. 616
 Eimiller, A. 95
 Eisele, K. 187
 Elten, H. 290
 Emmerich, B. 291
 Engberg, J. 312
 Epp, O. 614
 Erben, V. 291
 Erdmann, V. A. 301, 304
 Ey, P. 253

 Faber, O. K. 751
 Faerber, P. 313
 Falke, D. 278
 Fanning, E. 339
 Fantes, K. H. 121
 Fasiolo, F. 292
 Faulhammer, H. G. 292
 Fehlhammer, H. 614
 Feix, G. 313, 332
 Feldmann, H. 297
 Femfert, U. F. 233
 Ferber, E. 253
 Feuth, H. 153
 Fields, D. A. 318
 Figura, K. v. 268, 272, 641
 Filjak, B. 264, 493
 Fischer, G. 201
 Fischer, H. 619
 Fittler, F. 314
 Flemming, C. 629
 Floßdorf, J. 296, 299, 305
 Föhles, J. 741
 Forde, B. G. 259
 Frank, G. 585
 Franke, W. W. 314
 Freisl, M. 282
 Freist, W. 293
 Frischauf, A.-M. 315
 Fritz, H. 207, 401, 667, 855
 Fuchs, E. 321
 Fuchs, F. 252
 Fülling, R. 302
 Fujiki, H. 315

 Gabain, A. v. 308
 Gaertner, U. 280
 Ganbaryan, A. S. 305

- Gantner, G. 257
 Garel, J. P. 293
 Gaumert, R. 250
 Gehring, U. 496
 Geider, K. 309
 Geiger, Reinhard 553
 Geiger, Rolf 759, 825
 Gelbke, H. P. 75
 Gerok, W. 324
 Geyer, H. 257
 Ghosal, D. 316
 Giegé, R. 293
 Giesing, M. 258
 Glätzer, K. H. 316
 Gmeiner, J. 258
 Göbel, P. 269
 Goldmann, K. 256
 Golecki, J. 253
 Golf, S. W. 35
 Gorka, G. 117
 Gottikh, B. P. 319
 Govindan, M. V. 317
 Govindan, V. M. 496
 Graef, V. 35
 Graf, B. 492
 Graf, H. 294
 Greenberg, R. 333
 Greiling, H. 499
 Grenner, G. 887
 Grierson, D. 320
 Grieshaber, M. 259
 Gröbner, P. 259
 Grokhovsky, S. L. 319
 Groneberg, J. 317
 Gronenborn, B. 309
 Groner, B. 318, 337
 Gross, C. G. 318
 Gross, H. J. 301
 Grote, H. 317
 Grunicke, H. 257, 328
 Groß, P. 318
 Gschwendt, M. 318
 Guder, W. G. 259
 Günther, H. 268, 275
 Gunawan, J. 707
 Gunzer, G. 147
 Gurari-Rotman, D. 123
 Gursky, G. V. 319
 Gutensohn, W. 260

 Haar, F. v. d. 295, 819
 Haas, R. 319
 Hadváry, P. 266
 Hämmerling, G. 616
 Hagen, U. 310
 Hagenmaier, H. 273

 Hamlyn, P. H. 616
 Hamm, H. H. 492
 Hammer-Raber, B. 295
 Hantke, K. 260
 Harbers, E. 307
 Hartmann, A. 415
 Hartree, E. F. 57
 Hassels, B. 248
 Haupt, H. 435
 Hauser, H. 320
 Hegardt, F. G. 250
 Heger, H. W. 261
 Heger, I. 147
 Heidrich, H.-G. 253, 261
 Heinrich, P. C. 319
 Heldt, H. W. 249, 282
 Hell, R. 207, 855
 Helmreich, E. J. M. 262
 Hemleben, V. 320
 Hemminki, K. 1
 Hennecke, H. 296
 Henning, R. 621
 Henschen, A. 605
 Herrlich, P. 282, 328
 Hershey, H. V. 341
 Hess, M. 622
 Hess, U. 324
 Heuer, E. 325
 Hilfenhaus, J. 124
 Hilla, W. 153
 Hiltz, H. 51, 527
 Hinz, H.-J. 241, 263, 296, 305
 Hinze, H. 735
 Hirsch, F. 257
 Hirsch, R. 509
 Hirsch-Kauffmann, M. 328
 Hirth, K.-P. 321
 Hobom, G. 313, 316, 333
 Hochstraßer, K. 153, 253, 487
 Höller, M. 573
 Hörz, W. 321, 327
 Hoffmann, H. 291
 Hofmann, F. 263, 427
 Hofschneider, P. H. 307
 Holbrook, J. 623
 Hollandt, H. 307
 Holdorf, A. W. 268
 Holler, E. 295, 300
 Holzer, H. 727, 735
 Holzweber, F. 328
 Hood, L. 621

 Hoppe, J. 264
 Hoppen, H. O. 67
 Horak, I. 322
 Horst, J. 324
 Hoshino, J. 264, 493
 Huber, M. 260
 Huber, R. 435, 614
 Huber-Friedberg, W. 897
 Hustedt, H. 296
 Huttner, W. B. 492
 Hynes, N. 318, 337

 Igo-Kemenes, T. 327
 Innerhofer, A. 270

 Jacob, J. 609
 Jacob, R. 271
 Jaeger, E. 467
 Jaenicke, R. 241, 256, 263, 284
 Jahn, H. 260
 Jakobs, K. H. 265
 Janz, E. 323
 Jatzkewitz, H. 201
 Jeanneret, L. 867
 Jeck, R. 287
 Jeep, S. 318
 Jennissen, H. P. 265
 Jerne, N. K. 622
 Jilek, G. 307
 Jungblut, P. W. 496
 Junger, E. 265
 Jungermann, K. 359
 Jungwirth, C. 125
 Jušić, M. 735
 Just, W. 266

 Kabat, E. A. 613
 Kadenbach, B. 266
 Kaerlein, M. 322
 Kafiani, C. A. 322
 Kahan, M. 302
 Kahle, P. 297
 Kalbacher, H. 269
 Kapitsa, E. L. 322
 Kappus, H. 351
 Karges, H. E. 124
 Katz, N. 359
 Katze, J. R. 301
 Kaul, S. 497
 Keilich, G. 283
 Keith, G. 293
 Kemper, B. 323
 Kempfle, M. 267
 Keppler, D. 248, 267, 281

 Keradjopoulos, D. 268
 Kern, D. 293
 Kersten, H. 303
 Kersten, W. 306
 Khattab, M. 377
 Kickhöfen, B. 745
 Kido, R. 649
 Kiltz, H. H. 233
 Kim, M. A. 268
 Kindl, H. 163, 177, 393
 Kinne, R. 250, 272
 Kishida, T. 121
 Kittler, M. 51
 Klein, U. 268
 Kleine, R. 629
 Kleinow, W. 297
 Klingler, W. 269
 Kloetzl, P. 336
 Klotz, M. 323
 Kluge, F. 324
 Knobloch, K. 269
 Knöchel, W. 495
 Knof, S. 467
 Knopf, K. 329
 Knuppen, R. 67, 75, 81
 Kössel, H. 316, 333
 Koller, Th. 328
 Kolosov, M. N. 322
 Krämer, U. 280
 Kraus, E. 233
 Krauss, G. 297
 Krauss, S. 341
 Kresse, H. 268, 308
 Kröger, A. 270
 Kröger, H. 264, 297, 308, 493, 495, 811
 Krone, W. 492
 Kühn, A. 279
 Kühn, S. 316
 Kühne, U. 264, 493
 Kula, M.-R. 296, 298, 299, 305
 Kunau, W. H. 255, 284
 Kunz, W. 324
 Kupfer, S. 769

 Lachmann, H. 270
 Lacko, I. 313
 Lauppe, H. F. 324
 Leberman, R. 543
 Lehrach, H. 325
 Leising, H. 271
 Lempart, K. 153
 Lengyel, H. 51
 Letsch, R. 265
 Lezius, A. 325

- Lichtner, R. 287
 Liebl, A. 415
 Liefländer, M. 783
 Limburg, K. 301
 Lindner, E. 825
 Linzen, B. 41
 Löffler, M. 275
 Löwel, M. 252
 Lorenz, S. 304
 Lottspeich, F. 605
 Lotz, B. 251, 777
 Ludwig, B. 177, 393
- Maass, G. 297, 301
 Machicao, F. 337
 Machleidt, W. 239
 Mahal, G. 290
 Maier, V. 271
 Malchow, D. 273
 Mann, K. 553
 Manthey, K.-F. 124
 Mantieva, V. L. 326
 Markussen, J. 751
 Marquardt, W. 492
 Martin, H. H. 258
 Martin, R. 298
 Marutzky, R. 299
 Mattersberger, J. 337
 Maurer, W. 302
 Mayer, F. 839
 Mayer, H. 893
 Mayer, P. 299
 Mazur, G. 335, 481
 Medugorac, I. 271
 Meer, B. 326
 Meltzow, W. 741
 Mersmann, G. 272, 641
 Michaelis, G. 7, 21
 Michel, R. 415
 Milner, R. J. 621
 Milstein, C. 616
 Mischke, D. 336
 Möller, G. 618
 Molitor, H. 313
 Moll, R. 327
 Mraz, W. 201
 Müller, G. 147
 Müller, J. 673
 Müller, R. 267
 Müller, U. 257
 Müller, W. 310
 Müller-Hill, B. 309
 Müller-Wecker, H. 695
 Murawski, U. 288
 Murer, H. 250, 272
- Naithani, V. K. 751, 107
 Nanjundiah, V. 273
 Nass, G. 305
 Nath, A. 492
 Nebelin, E. 103
 Nees, S. 839
 Neuhoff, V. 225, 593
 Neupert, W. 415
 Nevers, P. 327
 Nicolai, A. D. v. 258
 Nicolai, H. v. 281, 287
 Nguyen, M. C. 318
 Noguchi, T. 649
 Nordmeyer, J.-P. 893
 Notbohm, H. 307
 Nowack, H. 601
 Nowack, J. 427
 Nowotny, E. 35
 Núñez de Castro, I. 727
- Obermeier, R. 759
 Oertel, G. W. 249
 Ogburn, C. A. 122
 Ogilvie, A. 290, 306
 Ohms, J.-P. 273
 Ortin, J. 333
- Pachmann, U. 300
 Palm, D. 274
 Palm, P. 315
 Palm, W. 614, 795, 799
 Paucker, K. 122
 Pecht, I. 615
 Peleteiro Rueda de
 Rivadulla, E. 117, 276
 Penniall, R. 623
 Pernis, B. 622
 Peter, H. W. 261
 Peters, F. 301
 Peters, H. H. 492
 Pette, D. 280
 Pfaender, P. 117, 276,
 327
 Pfeiffer, E. F. 271
 Pfeiffer, W. 327
 Pfennig-Yeh, M. 328
 Pfeuffer, T. 262, 289
 Pfleiderer, G. 377
 Philipp-Dormston, W. K.
 274
 Phizackerley, R. P. 614
 Pimmer, J. 300
 Pingoud, A. 301
 Poljak, R. J. 614
 Pongs, O. 301
 Ponta, H. 328
- Porter, R. R. 616
 Portmann, R. 328
 Postius, S. 275
 Potter, M. 618
 Preis, R. 291
 Püschner, R. 271
 Puschendorf, B. 257,
 328
- Raba, M. 301
 Rabbitts, T. H. 616
 Radsak, K. 329
 Rafael, J. 275, 289
 Rainey, P. M. 295
 RajBhandary, U. L. 301
 Rajewsky, K. 616
 Rak, B. 329
 Rambeck, B. 268
 Rambeck, W. 275
 Rao, G. S. 276, 573
 Rao, M. L. 276, 573
 Rastetter, J. 291
 Rauen, H. M. 280
 Ravdt, G. 328
 Reber, T. 341
 Reif, H. J. 327, 329
 Reinauer, H. 265
 Reitz, M. 342
 Remmer, H. 351
 Renkawitz, R. 330
 Renkawitz-Pohl, R. 330
 Reske, K. 621
 Reutter, W. 248
 Rieder, H. 276
 Rieke, E. 264
 Riesner, D. 297, 301,
 331
 Rigler, R. 291, 300
 Ring, K. 277
 Robertson, J. M. 302
 Rösen, P. 265
 Rogall, G. 330
 Rogozhin, S. V. 337
 Rohde, H. 601
 Rohrer, H. 331
 Roth, M. 277, 867
 Rüde, E. 620
 Rüdiger, H. 278
 Rüegg, U. T. 123
 Rueß, K. P. 783
 Rüterjans, H. 302
 Ruhenstroth-Bauer, G.
 897
- Sachsenmaier, W. 259
 Saedler, H. 327, 329
- Sängler, H. L. 331, 336
 Sako, Y. 271
 Salem, A. 897
 Salganik, R. I. 332
 Sano, H. 313, 332
 Sauer, G. 318
 Saul, F. 614
 Saur, W. 265
 Schachtschabel, D. O.
 271
 Schäfer, H.-J. 278
 Schäfer, K. P. 320
 Schäfer, U. 324
 Schaffrath, D. 499
 Scharf, R. 467
 Schartau, W. 41
 Schattenberg, P. J. 573
 Schatz, H. 271
 Schauer, R. 559, 839
 Scheer, U. 314
 Scheidtman, K. H. 333
 Scheit, K.-H. 338
 Scherer, G. 333
 Scherer, R. 897
 Scheulen, M. 351
 Scheurich, P. 278
 Schick, J. 339
 Schiebel, W. 334
 Schiefer, S. 163
 Schiemann, H. 683
 Schiffmann, D. 322
 Schilling, R. 342
 Schirmer, H. 249
 Schleicher, E. 535
 Schleuning, W.-D. 207,
 667, 855
 Schlimme, E. 290
 Schmid, F. 241, 263
 Schmid, W. 317, 494
 Schmidt, F. 334
 Schmidt, H.-L. 887
 Schmidt, W. 303
 Schmidtchen, F. P. 275
 Schneider, D. 278
 Schnell, E. 567
 Schneller, J. M. 298
 Schoelkens, B. A. 825
 Schönharting, M. 567
 Scholz, R. 279, 282
 Schou, O. 103
 Schrader, J. W. 621
 Schramm, H. J. 477
 Schriefers, H. 95
 Schriewer, H. 280
 Schudt, Ch. 280
 Schüttler, A. 741

- Schütz, G. 318, 335, 337, 495
 Schultze, G. 265
 Schulze, E. 225, 593
 Schumacher, G. 335
 Schwarz, E. 333
 Schweiger, A. 335, 481
 Schweiger, M. 328
 Schwenen, M. 254
 Schwenk, L. 274
 Schwick, G. 435
 Schwochau, M. 336
 Seela, F. 303
 Seelig, A. 280
 Seelig, J. 280
 Segger, H. 281
 Seif, F. J. 269
 Seifart, K. H. 308
 Seitz, H. J. 492
 Sekeris, C. E. 317, 494
 Sekita, T. 605
 Seng, P. N. 281
 Seubert, W. 492
 Shams Borhan, G. 667
 Shemijakin, M. F. 322
 Siebert, G. 567
 Simon, H. 268, 275, 535
 Singh, A. 336
 Sippel, A. E. 318, 337, 495
 Skryabin, K. G. 337
 Smith, D. F. 281
 Soboll, S. 282
 Sogo, J. M. 328
 Sonnenbichler, J. 337
 Sotomatsu, S. 121
 Spangenberg, P. 629
 Spoerel, N. 282
 Spring, H. 314
 Sprinzl, M. 301, 303, 304
 Stahl, A. 298
 Stahl, J. 295
 Staib, W. 252, 254
 Standke, K.-H. C. 283
 Starlinger, P. 334
 Staudenbauer, W. L. 337
 Stein, H. 338
 Stein, W. D. 573
 Steinmetz, M. 338
 Stender, W. 338
 Stewart II, W. E. 124
 Still, J. 250
 Stingelin, J. 299
 Stocker, H. 459
 Stötzler, D. 255
 Stoffel, W. 7, 21, 127
 Stone, P. R. 51
 Streeck, R.-E. 339
 Stremmel, W. 803
 Strominger, J. L. 620
 Stubenrauch, G. 75, 81
 Stuhlsatz, H. W. 499
 Stukacheva, E. A. 322
 Suhr, N. 283
 Summ, H.-D. 683
 Sunshine, M. 315
 Tarnowski, W. 492
 Teifel, W. 163
 Thamm, P. 467
 Thiebe, R. 304
 Thiele, H.-G. 247
 Thiessen, K. 306
 Thomale, J. 305
 Tiedemann, H. 497
 Timpl, R. 601
 Tiryaki, D. 721
 Tjia, S. 339
 Tjong, H. B. 707
 Törmä, E. 122
 Tonegawa, S. 617
 Torff, H.-J. 284
 Träger, L. 251, 777
 Trendelenburg, M. 314
 Tsai, H. 298
 Tschesche, H. 139, 657, 667, 769
 Tumanyan, V. G. 319
 Uhlenbruck, G. 254
 Varlamov, V. A. 337
 Varshavsky, A. J. 340
 Veh, R. W. 559
 Venkstern, T. V. 305, 337
 Viehhauser, G. 125
 Villemoes, P. 713
 Vizethum, W. 313
 Völkl, W. 887
 Voelter, W. 269
 Vogt, H.-P. 107
 Volkmann, H.-D. 683
 Vosberg, H. P. 340
 Wachter, E. 239
 Wacker, A. 313, 497
 Wagle, S. R. 263
 Wagner, K. G. 264, 341
 Wagner, T. 275, 304
 Wallat, S. 284
 Warbinek, R. 605
 Warth, R. 745
 Wasner, H. K. 285
 Weber, K. W. 296, 305
 Wehling, K. 341
 Weiler, E. 622
 Weinert, M. 783
 Weingärtner, B. 342
 Weiss, G. 492
 Weissbach, A. 329
 Weitzel, G. 187
 Wember, M. 559
 Wendlberger, G. 447, 459
 Wenzel, B. 673
 Wenzl, S. 256
 Werner, D. 341
 Werner, E. 297
 Werner, G. 266
 Werner, M. 67
 Werries, E. 285
 Westphal, M. 333
 Wiebauer, K. 306
 Wieland, Th. 89
 Wigzell, H. 618
 Wilhelm, I. 324
 Wilkening, J. 427
 Willecke, K. 341
 Willmitzer, L. 309
 Winkler, H. 267
 Winnacker, E.-L. 342
 Wintermeyer, W. 291, 302
 Wintersberger, E. 327, 330
 Wissler, J. H. 286
 Wissmann, H. 825
 Witt, W. 287
 Woenckhaus, C. 287
 Woertz, G. 259
 Wolf, H. 257
 Wolf, H. U. 287
 Wollenberg, P. 351
 Wollmer, A. 107
 Wünsch, E. 447, 459, 467
 Wunderer, G. 239, 409
 Wurster, B. 288
 Young, B. 493
 Zachau, H. G. 509
 Zackor, J. 288
 Zaharyev, V. M. 337
 Zahn, H. 107, 741
 Zahn, R. K. 342
 Zasedatelev, A. S. 319
 Zech, K. 269
 Zenner, H.-P. 289
 Zhuze, A. L. 319
 Ziffer, J. A. 621
 Zillig, W. 315, 328, 331
 Zilliken, F. 258, 281, 287
 Zimmer, G. 289
 Zimmer, P. 279
 Zimmermann-Telschow, H. 695
 Zipper, P. 256
 Zöllner, E. J. 342
 Zuber, H. 585

Action of Intracellular Proteinases on Mitochondrial Translation Products of *Neurospora crassa* and *Schizosaccharomyces pombe*

Rainer MICHEL, Anton LIEBL, Anton HARTMANN and Walter NEUPERT

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München

(Received 9 December 1975)

Summary: Gel electrophoretic analysis of mitochondrial membranes from *Neurospora crassa* shows the presence of a polypeptide fraction with apparent molecular weights of 7 000 - 12 000, which is synthesized on mitochondrial ribosomes. This fraction comprises between 10 and 50% of total mitochondrial translation products. Evidence is presented that the major part of this fraction is derived from components with higher apparent molecular weights by proteolytic activity. The proteolytic activity is located in vesicles which are co-isolated with mitochondria upon differential centrifugation. The activity is strongly enhanced by application of detergents such as sodium dodecylsulfate and Triton. Proteins synthesized on mitochondrial as well as cytoplasmic ribosomes are subject to proteolytic breakdown. This proteolysis can be blocked by addition of

inhibitors such as diisopropylfluorophosphate to isolated mitochondria. Similar observations were made with *Schizosaccharomyces pombe*. In *Neurospora*, the amount of mitochondrial translation products with apparent molecular weights of less than 12 000 is low in mitochondria from cells treated with cycloheximide for 1 h and high in mitochondria from cells treated with cycloheximide for 5 min. This observation is explained by the finding that proteinase activity in mitochondrial preparations decreases exponentially with a $t_{1/2}$ of 20 min during preincubation of cells with cycloheximide. Procedures are described to remove or block contaminating proteinase activity. The results appear to be relevant for the interpretation of many data obtained from experiments in which this puzzling kind of artifact has not been sufficiently considered.

Wirkung intrazellulärer Proteasen auf mitochondriale Translationsprodukte von Neurospora crassa und Schizosaccharomyces pombe

Zusammenfassung: Gelelektrophoretische Analyse der mitochondrialen Membranen von *Neurospora crassa* zeigt die Anwesenheit einer Polypeptidfraktion mit scheinbarem Molekulargewicht von 7 000 - 12 000, die an den mitochondrialen Ribosomen gebildet wird. Bezogen auf die gesamten mitochondrialen Translationsprodukte, macht

diese Fraktion zwischen ca. 10 und 50% aus. Es wird gezeigt, daß der größte Teil dieser Fraktion aus Komponenten mit höheren scheinbaren Molekulargewichten durch proteolytische Aktivität entstanden ist. Die proteolytische Aktivität ist in Vesikeln lokalisiert, welche bei differentieller Zentrifugation zusammen mit den Mitochondrien

isoliert werden. Durch den Einsatz von Detergentien wie Natriumdodecylsulfat oder Triton wird die Proteaseaktivität stark erhöht. Sowohl Proteine, die an den mitochondrialen Ribosomen gebildet werden als auch solche, die an den cytoplasmatischen Ribosomen gebildet werden, sind von dem proteolytischen Abbau betroffen. Dieser Abbau kann durch Zugabe von Inhibitoren wie Diisopropylfluorophosphat zu den isolierten Mitochondrien geblockt werden. Ähnliche Beobachtungen wurden mit *Schizosaccharomyces pombe* gemacht. In *Neurospora* ist der Anteil der mitochondrialen Translationsprodukte mit apparenten Molekulargewichten von weniger als 12 000 niedrig in den Mitochondrien aus Zellen,

die 1 h mit Cycloheximid behandelt wurden, und hoch in Mitochondrien aus Zellen, die 5 min mit Cycloheximid behandelt wurden. Die Erklärung für diesen Effekt wird durch den Befund geliefert, daß die Proteaseaktivität in Mitochondrienpräparationen bei Vorinkubation der Zellen mit Cycloheximid exponentiell mit einer Halbwertszeit von ca. 20 min abnimmt. Es werden Verfahren beschrieben, um kontaminierende Proteaseaktivität zu entfernen oder zu inhibieren. Die Ergebnisse, die hier dargestellt werden, erscheinen relevant für die Interpretation einer Reihe von Ergebnissen aus Experimenten, in denen diese verwirrende Quelle von Artefakten nicht ausreichend beachtet wurde.

Key words: Mitochondrial translation products; intracellular proteinases, cycloheximide, *Neurospora*.

Investigations of cellular reactions involving the subfractionation of cells are often subject to experimental artifacts which are caused by the activation of intracellular degradative enzymes. This is especially relevant for proteolytic enzymes which become redistributed during cell disruption. In many types of cells, proteolytic enzymes are compartmented in special organelles such as lysosomes or proteinase vacuoles (for reviews see^[1,2]). These vesicles are often co-isolated with mitochondria and their hydrolytic activity may have serious damaging effects when reactions in mitochondria are studied after subfractionation of the organelles. We have been confronted with this kind of artifact in our studies on the synthesis and structure of mitochondrial translation products in *Neurospora crassa*. There are many reports in the literature which describe the occurrence of mitochondrial translation products with apparent molecular weights of less than 12 000^[3-14]. These low molecular weight products are found in addition to the well described high molecular weight mitochondrial translation products, which are components of the mitochondrial membrane complexes cytochrome oxidase, cytochrome b and ATPase^[15,16]. Several different explanations have been offered for the occurrence of the low molecular weight polypeptides^[3-18]. Generation of these polypeptides by a physiological or artifactual action of intracellular pro-

teinases was investigated as one possible explanation. In this report experiments are presented which suggest that these polypeptides are mainly generated by the artifactual action of intracellular proteinases.

Material and Methods

Growth conditions

Neurospora hyphae (wild-type 74 A re) were grown in aerated liquid cultures at 25 °C in Vogel's minimal medium^[19] supplemented with 2% sucrose. The inoculum was 5×10^5 conidia per ml. The cells were harvested after 18 h growth (mid-log phase) by filtration. *Saccharomyces cerevisiae* (D-1827 leu⁻) and *Schizosaccharomyces pombe* (32 h⁻ leu⁻) were grown in complete medium supplemented with 3% glycerol into log phase and harvested by centrifugation^[20].

Isolation of mitochondria and gradient centrifugation

Mitochondria from *Neurospora crassa* were prepared as described by Weiss et al.^[21], mitochondria from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* according to the method described by Balcavage and Mattoon^[22].

Centrifugation of mitochondrial preparations on linear sucrose gradients was performed for 1 h at 78 000 × g in a Beckman Spinco, rotor SW 25.2. Sucrose concentrations were 20 - 60% (w/v) in 2mM EDTA, 10mM Tris/HCl, pH 7.4.

Extraction of whole cells with sodium dodecylsulfate-containing buffer

0.1 - 0.2 g of *Neurospora* hyphae (wet weight) were transferred to a mortar, frozen with liquid nitrogen and ground under liquid nitrogen for 2 min. After evaporation of the nitrogen, 0.2 ml of 0.1M Tris/HCl, 1% sodium dodecylsulfate, pH 8.0, was added and grinding was continued at 0 °C for a further 2 min. The resulting slurry was transferred to centrifuge tubes and centrifuged for 4 min at 17 000 × g at 0 °C. The supernatant was collected, dialysed for 2 h against 0.1M Tris/HCl, 1% sodium dodecylsulfate, pH 8.0 at 4 °C and then subjected to gel electrophoresis.

Labeling procedures

After 16 h growth, [¹⁴C]L-leucine (spec. radioact. 254 mCi/mmol) (Radiochemical Centre, Amersham, England) was added to the cultures (50 μCi/l). Two h later, cycloheximide was added (0.1 g/l; C. Roth OHG, Karlsruhe, Germany) and after another 2.5 min [³H]L-leucine (spec. radioact. 53 Ci/mmol; NEN Chemicals GmbH, Dreieichenhain, Germany) was added (1 mCi/l). Further incubation was carried out for the time periods indicated in the individual experiments. At the end of incubation, cells were chilled and harvested by filtration or centrifugation.

Determination of protein and radioactivity

Protein was estimated with the biuret method^[23]. Procedures for determination of radioactivity and for performance of gel electrophoresis were as described before^[6].

Determination of proteinase activity

Proteinase activity was determined by following the hydrolysis of azo-casein according to the method described by Hazen^[24]. The test volume was reduced to 1 ml.

Results

Proteolytic degradation of mitochondrial proteins

Mitochondria were isolated from cells in which total cellular proteins were homogeneously labeled with [¹⁴C]leucine and mitochondrial translation products specifically labeled in the presence of cycloheximide with [³H]leucine for 5, 20 and 60 min. The radioactivity patterns obtained upon gel electrophoresis after dissolving in sodium do-

decylsulfate-containing buffer are shown in Fig. 1 a - c. After the 5 min labeling period, the ¹⁴C- und ³H-radioactivities are found in material essentially with molecular weights in the range of 7 000 to 15 000. After a labeling period of 20 min, more of the radioactivity is found associated with bands of higher molecular weights (20 000 - 80 000) and less radioactivity in the low molecular weight range (Fig. 1 b). When incorporation was allowed to proceed for 60 min in the presence of cycloheximide, the homogeneous label was found to be associated mainly with bands corresponding to molecular weights of 20 000 to 80 000. The cycloheximide resistant label shows definite bands with apparent molecular weights of 40 000, 30 000 and 20 000. Low molecular weight material is present only in minor amounts.

One possible explanation for this observation is that under the conditions of the experiment massive proteolytic breakdown of mitochondrial and cytoplasmic translation products takes place in mitochondria after short (5 min) but not after long (60 min) cycloheximide treatment of cells. In order to verify this explanation, samples of mitochondria from the same respective batches were first treated with the proteinase inhibitor diisopropylfluorophosphate (DFP) before dissolving the mitochondria in dodecylsulfate. Then the same procedure was followed as with the samples not treated with DFP. The result of this experiment is shown in Fig. 1 d - f. In all DFP treated samples irrespective of the period of incubation in the presence of cycloheximide, the homogeneous label (¹⁴C) displays mainly bands with high molecular weights and the pattern is quite similar to that seen in Fig. 1 c. Also, in the cycloheximide resistant (³H) labeling patterns of the three samples, the components with higher molecular weights are predominant. Low molecular weight material is only observed after 5 min incorporation in the presence of cycloheximide and the pattern appears somewhat diffuse. In this case, the inhibitory effect of DFP may not be complete and/or there may be some breakdown even before sodium dodecylsulfate is added.

When gels were stained with Coomassie Brilliant Blue a protein distribution was found which coincided with the homogeneous ¹⁴C label under all the different conditions described in Fig. 1.

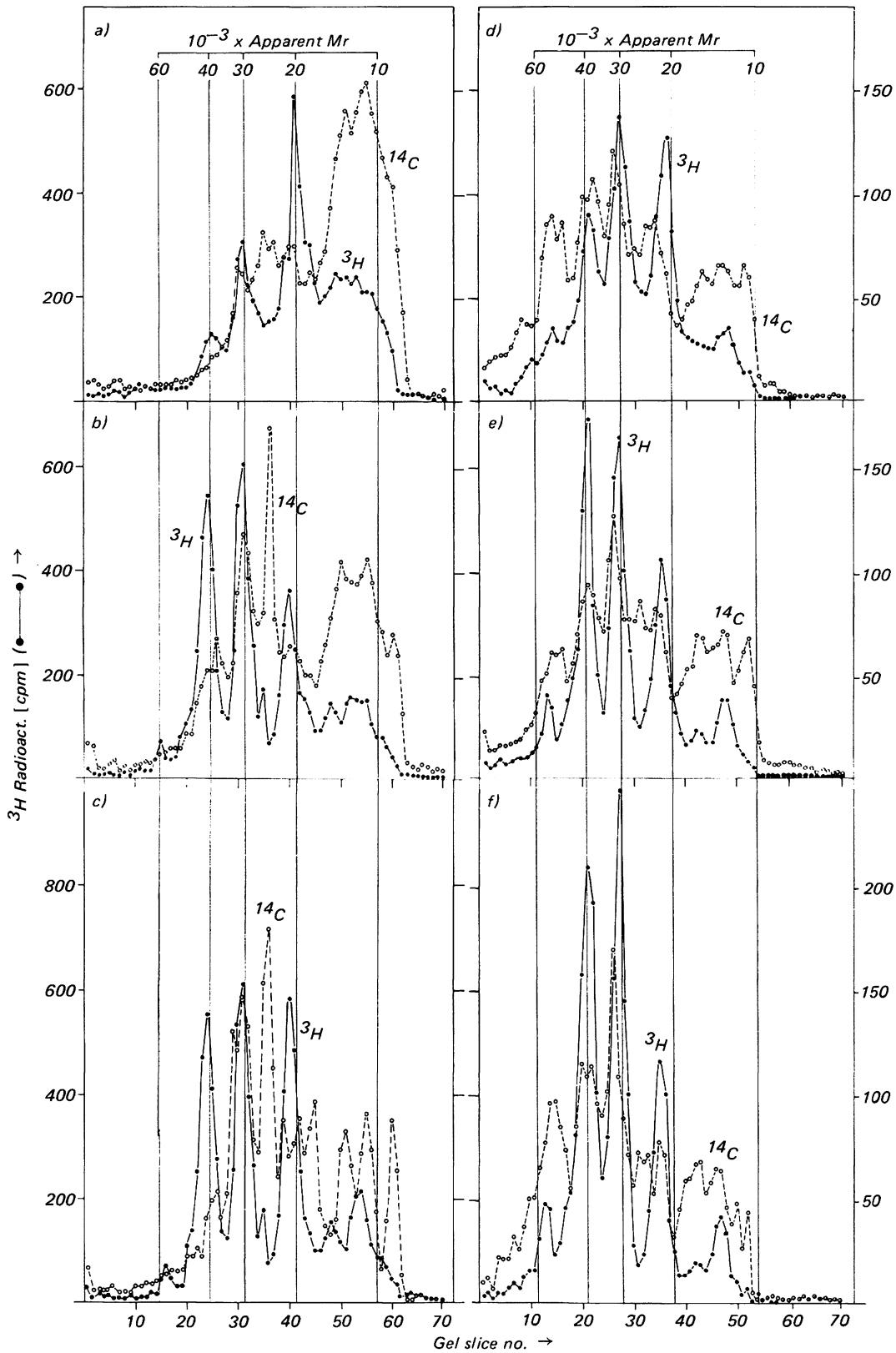




Fig. 1. Gel electrophoretic analysis of *Neurospora* mitochondrial proteins after different periods of labeling of cells with [^3H]leucine in the presence of cycloheximide.

Cells were homogeneously labeled with [^{14}C]leucine and specifically labeled with [^3H]leucine after addition of cycloheximide for 5 min (a, d), 20 min (b, e) and 60 min (c, f). Mitochondrial preparations were dissolved in sodium dodecylsulfate-containing buffer without adding DFP (a, b, c) (left column) and after adding DFP (1mM) (d, e, f) (right column). ○-----○ Homogeneous label (^{14}C); ●-----● specific (cycloheximide resistant) label (^3H).

These findings suggest:

- that preparations of *Neurospora* mitochondria contain proteolytic enzyme activity and that this activity decreases with increasing periods of cycloheximide treatment of cells;
- that proteolysis takes place after dissolving the mitochondria with sodium dodecylsulfate;
- that mitochondrial translation products are more resistant to proteinase than cytoplasmic translation products, especially those with apparent molecular weights of about 20 000.

In order to check whether activation of this proteolytic activity is also produced by Triton X-100, mitochondria from cells labeled homogeneously with [^{14}C]leucine as described for Fig. 1 and then labeled with [^3H]leucine for 15 min in the presence of cycloheximide were exposed to Triton X-100 under the following conditions (Fig. 2): First, as a reference, to one sample DFP was added and the mitochondria were kept for 1 h at 0 °C. Then Triton was added and immediately thereafter sodium dodecylsulfate. DFP was added to exclude proteolytic breakdown during the 1 h period and after dodecylsulfate treatment of mitochondria. Then gel electrophoresis was carried out (Fig. 2a). Both the ^{14}C - and ^3H labeling patterns indicate that no proteolytic breakdown occurred (cf. Fig. 1). When mitochondria were first dissolved in Triton X-100, kept for 1 h at 0 °C, 22 °C and 32 °C, then treated with DFP, dissolved in sodium dodecylsulfate and subjected to gel electrophoresis, radioactivity patterns were obtained which are shown in Fig. 2b, c and d. The ^3H pattern in Fig. 2b (0 °C) is similar to that of the reference (cf. Fig. 2a). The homogeneous label however shows an increase of material in the low molecular weight region. Treatment of mitochondria at 22 °C (Fig. 2c) gives rise to a considerable breakdown of the homogeneously labeled proteins. Also, the cycloheximide-resistant label shows degradation of mitochondrial

translation products. After incubation of mitochondria with Triton X-100 for 1 h at 32 °C (Fig. 2d), considerable quantities of both homogeneous and cycloheximide-resistant label are found in the low molecular weight range. Again, the cycloheximide-resistant label appears to be less affected compared to the homogeneous label. It is concluded from the data in Fig. 2 that after solubilisation of mitochondria with Triton X-100 breakdown of mitochondrially and cytoplasmically synthesized proteins does occur.

To determine whether proteinase also displays activity when mitochondria are kept in isolation medium, the following experiment was carried out. Mitochondria were incubated in the absence and presence of DFP in isolation medium for 30 min at 22 °C and then sodium dodecylsulfate gel electrophoresis was carried out. Mitochondrial translation products did not show indications of breakdown, however, cytoplasmic translation products with apparent molecular weights of 40 000 to 80 000 were broken down to a large extent.

Cellular localisation of proteinase activity

In order to test whether the proteolytic activity, effective in the experiments in Fig. 1 - 2, is associated with mitochondria or whether it just represents contaminating material, mitochondrial preparations were subjected to sucrose gradient centrifugation. After centrifugation mitochondria were seen as a turbid band in the lower third of the tube. Furthermore a pellet was obtained at the bottom of the tube. The gradient, divided into 10 fractions, and the pellet were monitored for protein content and for proteinase activity. The protein profile shows a peak at fract. 7. It represents mitochondria. About 10% of the total protein was found in the pellet fraction (Fig. 3a).

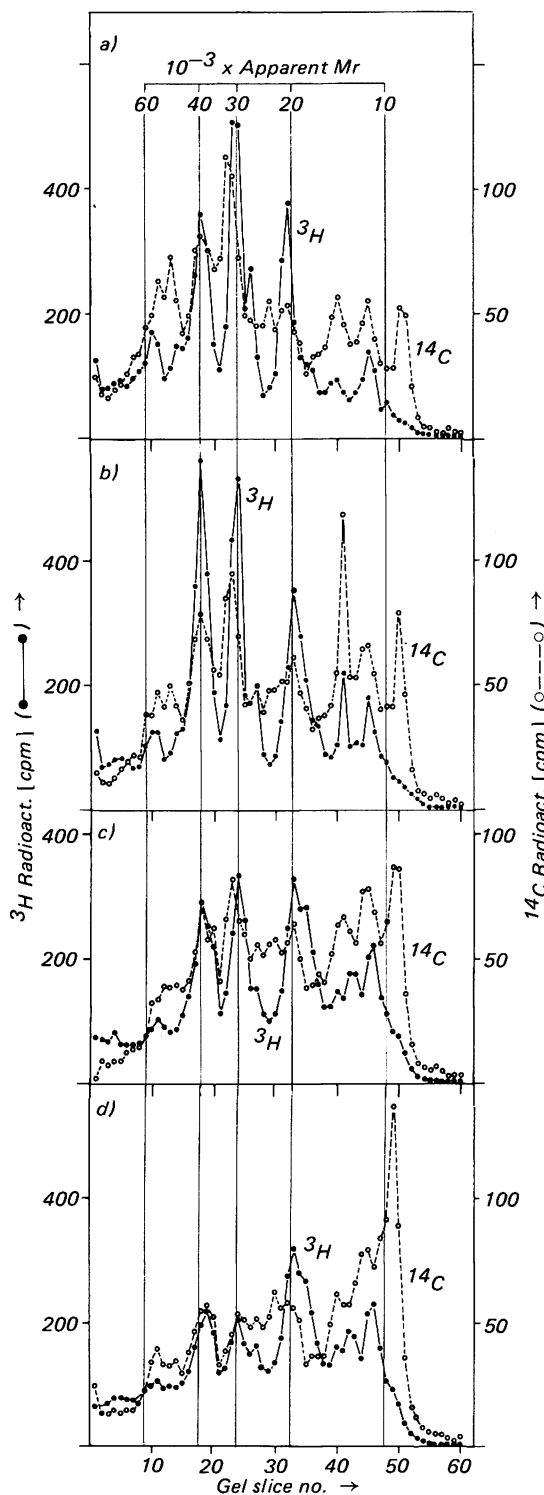


Fig. 2. Gel electrophoretic analysis of *Neurospora* mitochondrial proteins after solubilization with Triton X-100 under different conditions.

Cells were homogeneously labeled with [^{14}C]leucine and specifically labeled with [^3H]leucine for 15 min after addition of cycloheximide. The mitochondrial preparation was resuspended in 0.1 M Tris/HCl, pH 8.0, portions were treated with Triton X-100 under the following conditions and then subjected to gel electrophoresis.

a) DFP was added to a final concn. of 5mM and the sample incubated for 1 h at 0 °C. Triton X-100 and sodium dodecylsulfate were then added to final concns. of 1%, followed by additional DFP (1mM). b) Triton X-100 was added to a final concn. of 1% and the sample was incubated at 0 °C for 1 h. DFP was then added to a final concn. of 1mM, followed immediately by sodium dodecylsulfate to a final concn. of 1%.

c) Same as b) except that the incubation was carried out at 22 °C. d) Same as b) except that the incubation was carried out at 32 °C. ○-----○ Homogeneous label (^{14}C); ●-----● specific (cycloheximide resistant) label (^3H).

When assayed for proteolytic activity, virtually none was detected in the mitochondrial fraction but a high activity in the pellet fraction (Fig. 3b). This suggests that the proteolytic enzyme activity — at least that which is detected with this special test — is concentrated in particles which have a higher density than mitochondria. The existence of high density proteinase-containing vesicles in *Neurospora* has been described by Matile et al.^[25]. Moreover, Hasilik et al.^[26] have shown that proteinases A, B and C in *Saccharomyces cerevisiae* are mainly found in the vacuole fraction. When mitochondria were isolated from cells pretreated with cycloheximide for 1 h and subjected to the same centrifugation procedure, a practically identical protein profile was observed (Fig. 3a). Again no proteolytic activity was found in the mitochondrial fraction. In the pellet fraction, however, this activity related to the total amount of mitochondrial protein is much lower (about one fourth) if compared to preparations from cells not treated with cycloheximide (Fig. 3b). It is concluded from this observation that *Neurospora* cells in the presence of cycloheximide lose proteinase containing vesicles. This is in agreement with the findings presented in Fig. 1, that proteolytic breakdown of mitochondrial proteins is

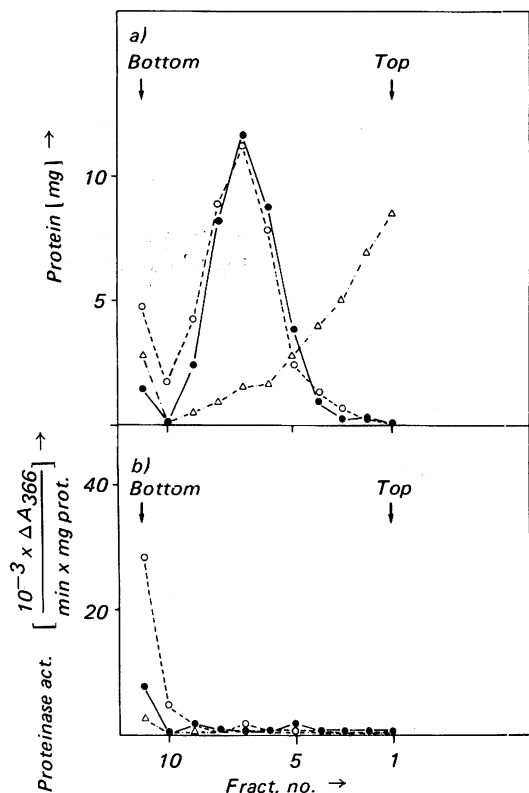


Fig. 3. Distribution of protein and proteinase activity in sucrose density gradient fractions after centrifugation of *Neurospora* mitochondrial preparations from cells treated in different ways.

The gradients were divided into 10 fractions and into the pellet fraction. \circ — \circ Cells grown for 18 h (control); \bullet — \bullet cells grown for 17 h, then treated with cycloheximide for 1 h; \triangle — \triangle cells grown for 18 h, then frozen with liquid nitrogen. a) Protein distribution; b) proteinase activity after solubilisation in Triton X-100.

much less expressed when cells from which mitochondria are isolated are pretreated with cycloheximide for 1 h.

In order to further prove that the degradative processes are actually caused by proteinase from contaminating vesicles, mitochondria were purified by density centrifugation. In this case, degradation after gel electrophoresis is strongly reduced with mitochondria from cells pretreated with cycloheximide for only 5 min.

A quite different way to remove the proteinase vesicles from mitochondrial preparations is suggested by the following experiment. After harvesting, *Neurospora* cells were rapidly frozen with liquid nitrogen and then thawed. Mitochondria were isolated as described for Fig. 1. Under these conditions, proteolytic breakdown after short and long treatment with cycloheximide is very low. An explanation for this effect is afforded by Fig. 3. Fig. 3a demonstrates that mitochondria isolated from cells frozen and thawed in this way do not form a distinct band upon sucrose gradient centrifugation. A considerable amount of protein, obviously representing broken mitochondria, remains at the top and partly smears to the bottom of the gradient. Determination of the proteinase activity in the fractions of the gradient reveals that in mitochondria from frozen cells practically no activity is found in the gradient fractions but that also in the pellet fraction proteinase activity is very low.

The simplest explanation for this effect is that in the freezing-thawing process not only mitochondria are disrupted but also the proteinase vesicles. The liberated proteinase is then removed from the mitochondrial membranes upon washing, which is carried out in the course of isolation of mitochondria. When proteinase activity is measured in a preparation of mitochondria resuspended in isotonic sucrose, a rather low activity is found. Furthermore, the activity increases during the test period. When mitochondria are lysed with Triton or subjected to sonication, the proteinase activity is several fold higher and no lag in the activity can be seen.

In the same test, sensitivity of the proteinase to various inhibitors was determined. The inhibitory effect of phenylmethylsulfonylfluoride and diisopropylfluorophosphate (final concentration 1mM) is about 90 to 95%. The sulfhydryl-group containing compound 2-mercaptoethanol (final concentration 10mM) is less effective, leaving 10 - 20% of the control activity uninhibited. The sulfhydryl inhibitor iodoacetamide (final concentration 1mM) does not inhibit the proteinase activity whereas the sulfhydryl sulfuric inhibitor *p*-chloromercuribenzoate (final concentration 1mM) causes a very strong inhibition of the proteinase activity (93%).

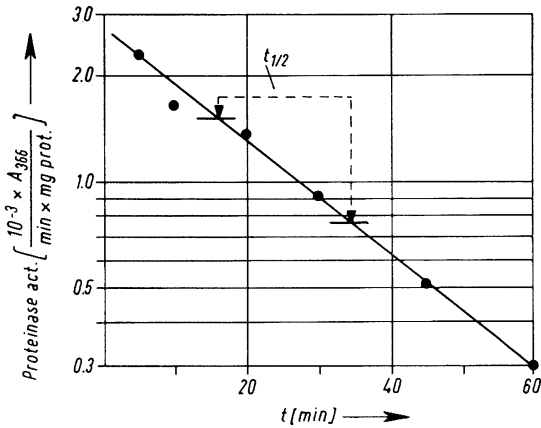


Fig. 4. Proteinase activity in *Neurospora* mitochondrial preparations obtained from cells treated with cycloheximide for various time periods.

Specific proteinase activity ($A_{366}/(\text{min} \times \text{mg protein})$) was determined after solubilizing mitochondria with Triton X-100. Abscissa: time of cycloheximide treatment.

Changes of proteinase activity during cycloheximide treatment of whole cells

Mitochondrial preparations were obtained from cells which were exposed to cycloheximide for increasing time periods up to 2 h. After solubilizing the mitochondrial preparations with Triton X-100, specific proteinase activity was determined (see Fig. 4). The activity was plotted on a logarithmic scale versus time of cycloheximide treatment. The straight line indicates an exponential decrease of proteinase activity in cells treated with cycloheximide. A half life of about 20 min can be calculated from these results.

Two conclusions may be drawn from these data:

- The proteinase which is detected by the in vitro test is subject to a rapid turnover. This turnover may either mean continuous synthesis and degradation of proteinase, or, more likely continuous synthesis and continuous excretion of the proteinase into the culture medium^[27].
- The decreasing degradation of mitochondrial and cytoplasmic translation products with increasing time of cycloheximide treatment of cells (cf. Fig. 1) appears to be an expression of the contamination of the mitochondrial preparation with proteinase vesicles.

Analysis of mitochondrial and cytoplasmic translation products without subfractionation of cells

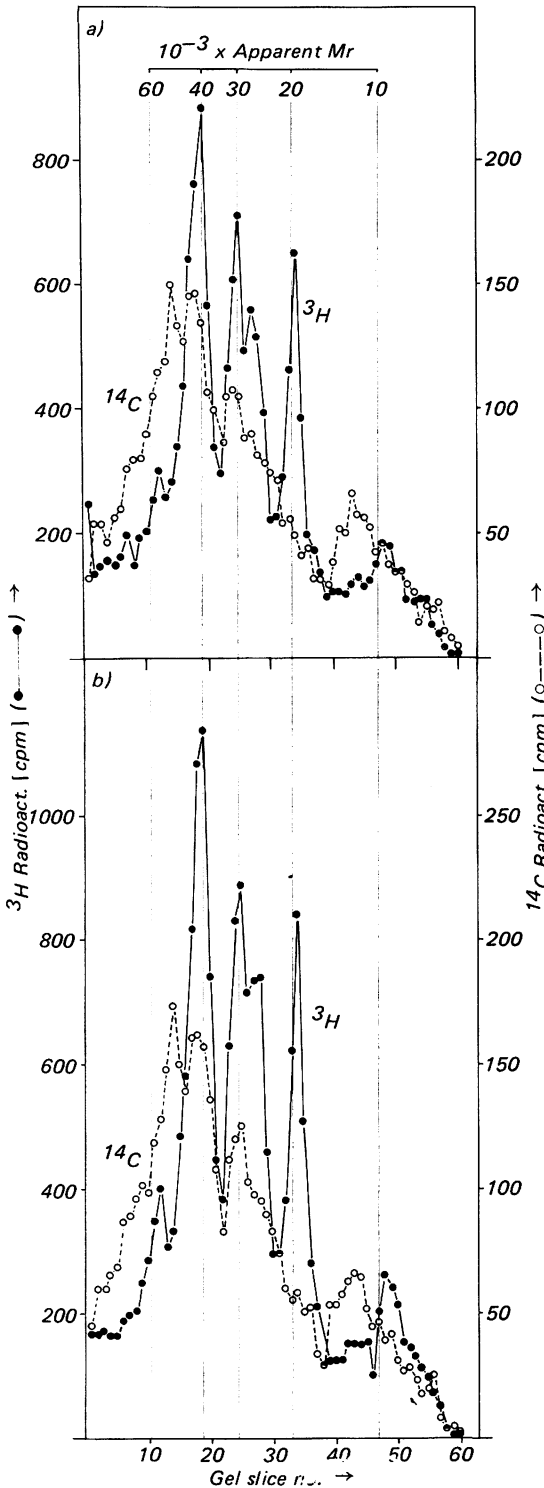
Since mitochondrial and cytoplasmic translation products can be selectively labeled it is possible to analyse them without separating cell fractions. This was done by simply freezing the cells with liquid nitrogen, breaking the cells by grinding at this temperature and extracting whole cellular proteins with a buffer containing sodium dodecyl-sulfate. A very similar procedure was described by Ebner et al. for yeast^[28]. The results after short term and long term labeling in the presence of cycloheximide are shown in Fig. 5. In this case, gel electrophoretic analysis shows practically identical homogeneous and specific labeling patterns with cells labeled for 5 and 60 min in the presence of cycloheximide. No proteinase inhibitor was applied in these experiments. Obviously, under the conditions of the experiment, the proteinase present in the cell is not active. This effect can be explained by the presence of a proteinase inhibitor in the cytoplasm of the cell. It inhibits the proteinase set free from the vacuoles. It must be assumed that upon isolation of mitochondrial fractions, the cytoplasmic inhibitor is removed and the proteinase can be active. The existence of proteinase inhibitors in *Neurospora* and yeast has actually been described^[27,29-31].

Comparison of *Neurospora* with yeast (*Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*)

In order to examine whether the degradation of proteins under the conditions described is a phenomenon which also occurs with other fungi, experiments similar to those described with *Neu-*

Fig. 5. Gel electrophoretic analysis of *Neurospora* mitochondrial translation products in extracts of whole cells. ▷

Cells were homogeneously labeled with [¹⁴C]leucine and specifically labeled with [³H]leucine in the presence of cycloheximide for 5 min (a) and 60 min (b). They were then frozen with liquid nitrogen. After breaking cells by grinding under liquid nitrogen, total cellular proteins were extracted with sodium dodecylsulfate-containing buffer, dialysed and subjected to gel electrophoresis. ○-----○ Homogeneous label (¹⁴C); ●-----● specific (cycloheximide-resistant) label (³H).



rospora were carried out with yeast. Yeast is widely employed for studies on the biogenesis of mitochondrial proteins. As with *Neurospora*, cells were homogeneously labeled with [^{14}C]leucine and specifically labeled in the presence of cycloheximide with [^3H]leucine. The mitochondrial fractions were isolated after breaking the cells with a glass bead homogenizer and gel electrophoresis was carried out after dissolving mitochondrial fractions in sodium dodecylsulfate without and with added DFP. The results with *Schizosaccharomyces pombe* are shown in Fig. 6. Without added DFP, ^{14}C - and ^3H radioactivities are broadly distributed over a molecular weight range of 8 000 to 45 000. Bands with definite molecular weights are not seen. If DFP is added before dissolving mitochondrial fractions in sodium dodecylsulfate, the cycloheximide-resistant label shows definite bands with apparent molecular weights between 20 000 and 40 000. In contrast to the sample not treated with DFP, the background is low and less radioactivity is seen at molecular weights of 8 000 to 20 000. The homogeneous label is distributed mainly between apparent molecular weights of 20 000 to 80 000. The electrophoretic pattern of mitochondrial translation products is very similar to that described by Ebner et al. for *Saccharomyces cerevisiae*^[28].

Further experiments with *Schizosaccharomyces* have shown that degradation is caused by proteinase activity which cannot be separated from mitochondria by sucrose density centrifugation. Furthermore, the proteinase activity appears not to be dependent on cycloheximide treatment of whole cells.

In the case of *Saccharomyces*, proteolysis of mitochondrial and cytoplasmic translation products was not observed under conditions which lead to the production of low molecular weight components in *Neurospora crassa* and *Schizosaccharomyces pombe*. Addition of DFP does not cause significant changes of the labeling patterns. Probably, the rather crude procedure for breaking the yeast cells leads to disruption of vacuoles and removal of proteinases during the isolation procedure of mitochondria. It might well be that under different conditions, such as isolation of mitochondria after preparation of spheroplasts, vacuoles are preserved and show degrading activity during subfractionation of mitochondria.

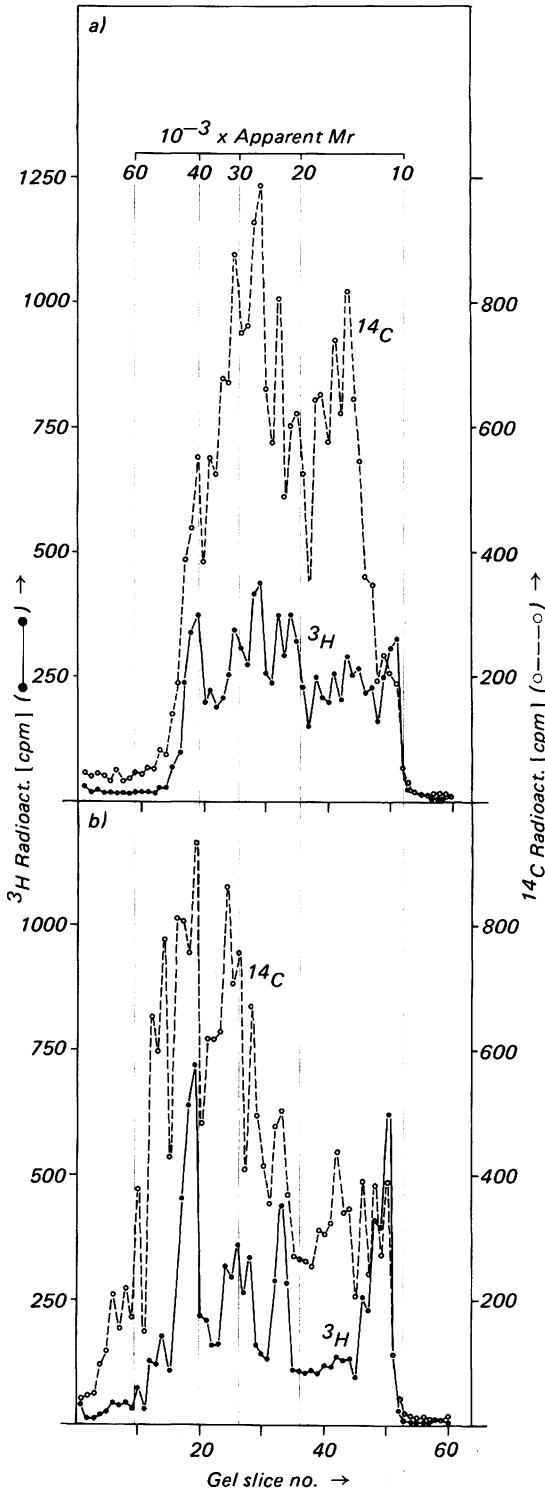


Fig. 6. Gel electrophoretic analysis of *Schizosaccharomyces pombe* mitochondrial proteins after solubilization with sodium dodecylsulfate in the presence or absence of proteinase inhibitor (DFP).

Cells were homogeneously labeled with [^{14}C]leucine for 1.5 h and specifically labeled with [^3H]leucine in the presence of cycloheximide for 15 min. Samples of the mitochondrial preparations were dissolved in sodium dodecylsulfate-containing buffer without (a) and with added DFP (1mM) (b) and subjected to gel electrophoresis. \circ — \circ Homogeneous label (^{14}C); \bullet — \bullet specific (cycloheximide resistant) label (^3H).

Discussion

This study on proteolytic activities associated with mitochondrial preparations from *Neurospora* and yeast was undertaken to clarify the significance of mitochondrial translation products with apparent molecular weights of less than 12000. The data obtained strongly suggest that under a variety of conditions the major part of the low molecular weight translation products is generated by the artifactual action of proteinases. The proteolytic activity which may represent several different enzymes^[32] is located in proteinase vesicles which are isolated together with mitochondria upon differential centrifugation. The activity becomes apparent when vesicles are opened. Breakdown of mitochondrial proteins therefore occurs essentially during procedures which involve the use of detergents. Disturbing effects of contaminating proteinases upon gel electrophoresis on isolated proteins or organelles from microorganisms have already been reported^[10,33]. In the experiments described here, the critical steps are those after solubilization of the mitochondria with sodium dodecylsulfate or Triton for the further subfractionation of mitochondrial membranes, especially by gel electrophoresis. Mitochondrial translation products appear to be less affected compared to cytoplasmic translation products.

As described earlier, the proportion of low molecular weight mitochondrial translation products is high, if mitochondria are isolated from cells which are not, or only for short time, exposed to cycloheximide, and low in mitochondria from cells exposed to cycloheximide for 1 h and more^[6]. This

observation led to some confusion. As a possible explanation we suggested that low molecular weight translation products are converted to high molecular weight translation products in the presence of cycloheximide^[6,34]. Similar observations and considerations were made by others, partly on the basis of different experimental approaches^[3,10,12,18]. The data presented in this report show that the interpretation is not correct, at least not for the results from experiments with cells exposed to cycloheximide for different periods. The real cause for the apparent shift of molecular weights from low to high during cycloheximide treatment is the decrease of proteinase activity. *Neurospora* cells grown on minimal medium into the early log phase possess proteinase vesicles which may represent proteinase storage and transport organelles. They contain proteinases probably destined for export into the extracellular space^[27]. Apparently the inhibitor of cytoplasmic protein synthesis, cycloheximide, blocks the production of new vesicles and the existing ones leave the cell with a half life of about 20 min, which is about 1/12 of the doubling time of the cells. It remains to be checked whether the artifacts resulting from proteinase activity play a similar role in the experiments of other authors, in which large amounts of low molecular weight translation products were observed and which led to speculations on a conversion of these products.

The variation of proteinase activity in cells treated with cycloheximide is just one example of the rather disturbing and not easily comprehensible effects of unspecific proteinases. Slight differences in the preparation of cell fractions may lead to the removal of proteinase vesicles to different degrees. After employment of detergents for the separation of membrane proteins, activation of proteinases may occur to varying extents. Action of cytoplasmically located inhibitors may lead to confusing results in experiments which are slightly different in their design. Cells in different phases of growth may have different levels of proteolytic enzymes.

In order to avoid artifacts, the application of suitable proteinase inhibitors such as diisopropylfluorophosphate or phenylmethylsulfonylfluoride appears to be very useful. However, their efficiency must be established for the individual experimental conditions. e.g. phenylmethylsulfonyl-

fluoride does not eliminate proteinase activity if it is just added during the isolation of mitochondria.

The observations described here may be of general importance for a broad variety of microorganisms which possess proteinase vacuoles and which are able to secrete proteinases into the extracellular space. Moreover, in higher organisms lysosomes may play a similar role^[35]. Furthermore, there may be large differences between closely related organisms, as described here for *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

The authors are grateful to A. Haid, Institut für Genetik der Universität München, for supplying the yeast strains *Saccharomyces cerevisiae* D-1827 leu⁻ and *Schizosaccharomyces pombe* 32 h⁻ leu⁻.

This investigation was supported by the Deutsche Forschungsgemeinschaft (Schwerpunktprogramm „Biochemie der Morphogenese“).

Literature

- 1 Matile, Ph. (1969) in *Lysosomes in Biology and Pathology* (Dingle, J. T. & Fell, H. B., eds.) Vol. 1, pp. 416 - 430, North-Holland Publ. Co., Amsterdam.
- 2 Tappel, A. L. (1969) in *Lysosomes in Biology and Pathology* (Dingle, J. T. & Fell, H. B., eds.) Vol. 2, pp. 207 - 244, North-Holland Publ. Co., Amsterdam.
- 3 Tzagoloff, A. & Akai, A. (1972) *J. Biol. Chem.* **247**, 6517 - 6523.
- 4 Hadvary, P. & Kadenbach, B. (1973) *Eur. J. Biochem.* **39**, 11 - 20.
- 5 Kuzela, St., Kolarov, J. & Krempasky, V. (1973) *Biochem. Biophys. Res. Commun.* **54**, 9 - 16.
- 6 Michel, R. & Neupert, W. (1973) *Eur. J. Biochem.* **36**, 53 - 67.
- 7 Wheeldon, L. W., Dianoux, A. C., Bof, M. & Vignais, P. V. (1974) *Eur. J. Biochem.* **46**, 189 - 199.
- 8 Burk, J. P. & Beattie, D. S. (1974) *Arch. Biochem. Biophys.* **164**, 1 - 11.
- 9 Küntzel, H. & Blossey, H.-C. (1974) *Eur. J. Biochem.* **47**, 165 - 171.
- 10 Lansman, R. A., Rowe, M. J. & Woodward, D. O. (1974) *Eur. J. Biochem.* **41**, 15 - 23.
- 11 Küntzel, H., Igbal, A. & Blossey, C. (1974) in *Biogenesis of Mitochondria* (Kroon, A. H. & Saccone, C., eds.) pp. 71 - 78, Academic Press, New York.
- 12 Hawley, E. S. & Greenawalt, J. W. (1975) *Eur. J. Biochem.* **54**, 585 - 601.
- 13 Küntzel, H., Pieniazek, N. J., Pieniazek, D. & Leister, D. E. (1975) *Eur. J. Biochem.* **54**, 567 - 575.
- 14 Michel, R., Liebl, A., Machleidt, W., Otto, J. & Neupert, W. (1975) *this J.* **365**, 1595 - 1604.
- 15 Schatz, G. & Mason, T. L. (1974) *Annu. Rev. Biochem.* **43**, 51 - 87.

- 16 Tzagoloff, A., Rubin, M. S. & Sierra, M. F. (1973) *Biochim. Biophys. Acta* **301**, 71 - 104.
- 17 Rücker, A. v., Michel, R. & Neupert, W. (1973) *this J.* **354**, 1237.
- 18 Kuzela, St., Krempasky, V., Kolarov, J. & Ujházy, V. (1975) *Eur. J. Biochem.* **58**, 483 - 491.
- 19 Vogel, H. J. (1956) *Microb. Genet. Bull.* **13**, 42 - 48.
- 20 Wolf, K., Sebald-Althaus, M., Schweyen, R. J. & Kaudewitz, F. (1971) *Mol. Gen. Genet.* **110**, 101 - 109.
- 21 Weiss, H., Jagow, G. v., Klingenberg, M. & Bücher, Th. (1970) *Eur. J. Biochem.* **14**, 75 - 82.
- 22 Balcavage, W. X. & Mattoon, J. R. (1968) *Biochim. Biophys. Acta* **153**, 521 - 530.
- 23 Beisenhertz, G., Boltze, H. J., Bücher, Th., Czock, R., Garbade, K. H., Mayer-Arendt, E. & Pfeleiderer, G. (1953) *Z. Naturforsch.* **8b**, 555 - 577.
- 24 Hazen, G. G. (1974) in *Methoden d. Enzymat. Analyse* (Bergmeyer, H. U., ed.) pp. 1038 - 1044, Verlag Chemie, Weinheim/Bergstr.
- 25 Matile, Ph. (1964) *Naturwissenschaften* **51**, 489 - 490.
- 26 Hasilik, A., Müller, H. & Holzer, H. (1974) *Eur. J. Biochem.* **48**, 111 - 117.
- 27 Matile, Ph. (1965) *Z. Zellforsch.* **65**, 884 - 896.
- 28 Ebner, E., Mennucci, L. & Schatz, G. (1973) *J. Biol. Chem.* **248**, 5360 - 5368.
- 29 Yu, P. H., Kula, M. R. & Tsai, H. (1973) *Eur. J. Biochem.* **32**, 129 - 135.
- 30 Tsai, H., Tsai, J. H. J. & Yu, P. H. (1973) *Eur. J. Biochem.* **40**, 225 - 232.
- 31 Betz, H., Hinze, H. & Holzer, H. (1974) *J. Biol. Chem.* **249**, 4515 - 4521.
- 32 Siepen, D., Yu, P. H. & Kula, M. R. (1975) *Eur. J. Biochem.* **56**, 271 - 281.
- 33 Pringle, J. R. (1970) *Biochem. Biophys. Res. Commun.* **39**, 46 - 52.
- 34 Michel, R. & Neupert, W. (1974) in *The Biogenesis of Mitochondria* (Kroon, A. H. & Saccone, C., eds.) pp. 315 - 326, Academic Press, New York.
- 35 Wieland, O. H. (1975) *FEBS Lett.* **52**, 44 - 47.

Dr. R. Michel, A. Liebl and PD Dr. Dr. W. Neupert, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität,
Goethestr. 33, D-8000 München 2;
A. Hartmann, Lehrstuhl Mikrobiologie II der Universität Tübingen,
Auf der Morgenstelle, D-7400 Tübingen.