[71] B. Giese, S. Wessely, M. Spormann, U. Lindermann, E. Meggers, M. E. Michel-Beyerle, *Angew. Chem.* **1999**, *111*, 1050 – 1052; *Angew. Chem. Int. Ed.* **1999**, *38*, 996 – 998.
[72] E. Meggers, D. Kusch, M. Spichty, U. Wille, B. Giese, *Angew. Chem.* **1998**, *110*, 474 – 476; *Angew. Chem. Int. Ed.* **1998**, *37*, 460 – 462.
[73] D. T. Breslin, G. B. Schuster, *J. Am. Chem. Soc.* **1996**, *118*, 2311 – 2319.
[74] P. T. Henderson, D. Jones, G. Hampikian, Y. Kan, G. B. Schuster, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8353 – 8358.
[75] D. J. Hurley, Y. Tor, *J. Am. Chem. Soc.* **1998**, *120*, 2194 – 2195.
[76] S. I. Khan, A. E. Beilstein, M. W. Grinstaff, *Inorg. Chem.* **1999**, *38*, 418 – 419.
[77] S. I. Khan, M. W. Grinstaff, *J. Am. Chem. Soc.* **1999**, *121*, 4704 – 4705.
[78] B. P. Hudson, J. K. Barton, *J. Am. Chem. Soc.* **1998**, *120*, 6877 – 6888.

Tyrosyl – tRNA Synthetase: A Housekeeping Protein and an Attractive Harbinger of Cellular Death

Hermann Schluesener*

Apoptosis, or programmed cell death, is a physiological process that occurs during tissue reorganization in embryonic development and pathological conditions. During execution of this cellular suicide program, nucleases fragment chromosomal DNA and activated proteases dismantle the cell by cleaving multiple substrates, including cytoskeletal proteins and enzymes that are essential for normal cellular function and cell repair.

Cells undergoing apoptosis are usually swiftly ingested by macrophages and this phagocytic clearance of intact apoptotic cells is a safe disposal route since local inflammatory reactions that result from a massive release of cellular debris are avoided. Furthermore, the ingestion of apoptotic cells by phagocytes may actively down-regulate local inflammatory and immune responses.[1]

Surprisingly, two novel signaling molecules of the apoptotic cascade have been described recently by Wakasuki and Schimmel.[2] These cytokines are fragments of tyrosyl – tRNA synthetase, an unsuspicious enzyme that is essential in protein synthesis (and consequently belongs to the housekeeping proteins). Cellular protein synthesis relies on a supramolecular organization of the mammalian translation system.[3] This protein-synthesizing machinery is a complex, but highly organized apparatus and the macromolecular components are not freely diffusible in mammalian cells. In particular, aminoacyl – tRNA appears to be channeled within the cell, that is, directly transferred from the aminoacyl – tRNA synthetases to the elongation factor, and then to the ribosomes, without dissociation into the cellular fluid.[4]

Functionally, human tyrosyl – tRNA synthetase is composed of a catalytic terminal amino domain and a terminal carboxy sequence. Approximately 50% of the terminal carboxy sequence is identical to the terminal carboxy region of the p43 protein, an auxiliary RNA binding factor of the mammalian multi-synthetase complex.[5]

[*] Prof. Dr. H. Schluesener
Institut für Hirnforschung der Universität
Calwer Strasse 3, D-72076 Tübingen (Germany)
Fax: (+49) 7071-29-5456
E-mail: hirnforschung@uni-tuebingen.de

During apoptosis, the translational apparatus is systematically destroyed. Interestingly, tyrosyl – tRNA synthetase is secreted from cells undergoing apoptosis. After cleavage by elastase or related proteases, two fragments with surprisingly novel bioactivities are generated (Figure 1). Both fragments

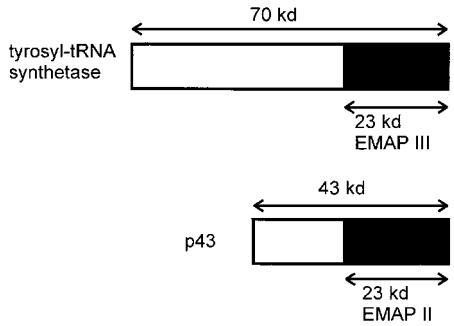

Figure 1. Tyrosyl – tRNA synthetase and the auxiliary p43 protein are cleaved by elastase or related proteases (such as caspases or multicatalytic protease) into two fragments. The terminal carboxy fragments EMAP II and III are homologous modulators of leukocyte migration and activation.

appear to act as local signaling molecules (cytokines) that orchestrate the tissue response and culminate in the safe removal of apoptotic cells.

Tyrosyl – tRNA Synthetase: Surprising Bioactivities of Cleavage Products

Full-length tyrosyl – tRNA synthetase has no effect on leukocytes, but both fragments are potent leukocytic activators with different spectra of bioactivities.[2] The catalytic aminoterminal domain contains an Glu-Ag-Leu (ERL) sequence motif, which is characteristic of certain chemokines, a family of chemoattractant cytokines. It has been shown that the aminoterminal peptide of tyrosyl – tRNA synthetase binds to the interleukin-8 (IL-8) receptor type A (also known as CXCR1). Subsequent bioassays showed that this peptide has IL-8 activity, predominantly by activating and inducing migration of neutrophilic granulocytes.[2]

  1433-7851/99/3824-3635 $ 17.50+.50/0

The carboxyterminal sequence is homologous to the carboxyterminal region of the protein p43. This fragment of p43 is likewise liberated during apoptosis and has cytokine-like properties. The cytokine is termed endothelial-monocyte-activating polypeptide II (EMAP II).[6] EMAP II and the terminal carboxy fragment of tyrosyl–tRNA synthetase, consequently designated EMAP III, have similar effects on leukocytes, but the receptors are not known and a comparative analysis of these two cytokines have not so far been performed.[7]

EMAP II and III activate resting monocytes by elevating the free Ca^{2+} concentration in the cytosol, thereby stimulating chemotaxis.[6] In contrast to the terminal amino fragments, the EMAPs stimulate the production of tumor necrose factor α (TNF-α) and tissue factor (thromboplastin) by macrophages and release of myeloperoxidase from neutrophilic granulocytes. Furthermore, EMAP II activates endothelial cells by elevation of the concentration of free calcium in the cytosol, release of von Willebrand factor, induction of tissue factor, and expression of the adhesion molecules E-selectin and P-selectin. EMAP II induces endothelial cell apoptosis, which results in the hemorrhage of blood vessels of certain experimental tumors, such as fibrosarcoma, melanoma, and adenocarcinoma, thereby inducing tumor regression. It is so far unknown, whether the terminal carboxy fragment of EMAP III has similar effects on endothelial cells.

Tyrosyl–tRNA Synthetase: A Novel Regulator of Tissue Homeostasis?

EMAP II is produced in tumors, neuroautoimmune and other inflammatory lesions, and during tissue reorganization in embryology.[8] In all of these conditions a disturbance in tissue homeostasis is associated with apoptosis and expression of EMAPs. Thus, a global regulatory mechanism is emerging, which links the degradation of the cellular translational machinery with homeostasis of the entire surrounding tissue elements. The process is summarized in Figure 2: tyrosyl–tRNA synthetase can be secreted by cells undergoing apoptosis and the secretion of an essential component of the translational apparatus might accelerate the cellular suicide program. Outside of the cell, the synthetase is split into two distinct signaling molecules. These cytokines are chemoattractants, which recruit neutrophilic granulocytes and phagocytes into the lesions. Furthermore, these activated leukocytes secrete proteases, which induces further generation of cytokines from tyrosyl–tRNA synthetase and invasion by leukocytes. Finally, this recursive cycle leads to removal of the apoptotic cells.

Outlook

The characterization of the exceptional organization of human tyrosyl–tRNA synthetase opens a variety of possibilities for further research. In addition to many basic aspects of phylogenetic development and function of these modules in other organisms, applied pharmacological research appears to be rewarding. Most prominently, the two cytokines with profound function on neutrophils and monocytes might be used as immunmodulators. In comparison to EMAP II, the terminal carboxy fragment of tyrosyl–tRNA synthetase might have effects on endothelial cells. EMAP II expression has been repeatedly linked with tumors: Mouse EMAP II has been cloned from fibrosarcoma and human EMAP II from histiocytoma cells. EMAP II appears to be identical to other previously described mediators of tumor cells, such as the cytokine J82 derived from bladder carcinoma (BCDC), or FO-1 and HS-1 produced by melanomas.[9] Thus, the terminal carboxy fragments of tyrosyl–tRNA synthetase and EMAP II have potential as anti-angiogenesis agents in tumor therapy, whereby they inhibit pathological tumor endothelial cell function and thereby promote thrombohemorrhage of tumor blood vessels associated with tumor regression. As a mediator associated with tumor angiogenesis, these peptides are highly interesting candidates of antiangiogenic drug development and might complement current strategies to abrogate tumor growth and metastasis by interfering with pathological endothelial cell function in neoangiogenesis.[10]

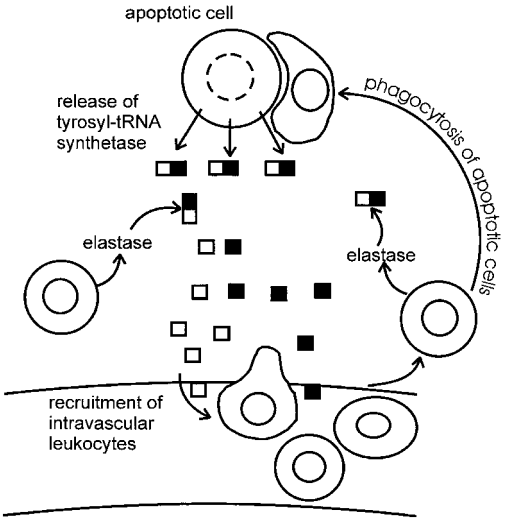

Figure 2. Tyrosyl–tRNA synthetase is secreted by apoptotic cells and split by elastase into two chemoattractant fragments, which recruit leukocytes from the vascular lumen into the tissue. The migrating leukocytes release further elastase, thus amplifying the proteolytic cleavage of tyrosyl–tRNA synthetase and the generation of chemoattractants. Finally, this self-amplifying, recursive cycle is terminated by safe removal of the apoptotic cells by invading macrophages.

German version: *Angew. Chem.* **1999**, *111*, 3852–3854

Keywords: antitumor agents · apoptosis · drug research · enzymes · tumor therapy

[1] a) Y. Ren, J. Savill, *Cell Death Differ.* **1998**, *5*, 563–568; b) V. J. Kidd, *Annu. Rev. Physiol.* **1998**, *60*, 533–573.
[2] a) K. Wakasugi, P. Schimmel, *Science* **1999**, *284*, 147–150; b) K. Wakasugi, P. Schimmel, *J. Biol. Chem.* **1999**, *274*, 23155–23159.
[3] a) B. S. Negrutskii, R. Stapulionis, M. P. Deutscher, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 964–968; b) R. Stapulionis, M. P. Deutscher, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7158–7161; c) M. T. Norcum, *FEBS Lett.* **1999**, *447*, 217–222.
[4] a) C. Francklyn, K. Musier-Forsyth, S. A. Martinis, *RNA* **1997**, *3*, 954–960; b) E. V. Koonin, L. Aravind, *Curr. Biol.* **1998**, *8*, R266–R269; c) S.

  1433-7851/99/3824-3636 $ 17.50+.50/0

Cusack, *Curr. Opin. Struct. Biol.* **1997**, *7*, 881 – 889; d) J. I. Sagara, S. Shimizu, T. Kawabata, S. Nakamura, M. Ikeguchi, K. Shimizu, *Nucleic Acids Res.* **1998**, *26*, 1974 – 1979.

[5] a) S. Quevillon, F. Agou, J. C. Robinson, M. Mirande, *J. Biol. Chem.* **1997**, 272, 32573 – 32579, b) T. A. Kleeman, D. Wie, K. L. Simpson, E. A. First, *J. Biol. Chem.* **1997**, 272, 14420 – 14425.

[6] a) J. Kao, K. Houck, Y. Fan, I. Haehnel, S. K. Libutti, M. L. Kayton, T. Grikscheit, J. Chabot, R. Nowygrod, R. S. Greenberg, W. J. Kuang, D. W. Leung, J. R. Hayward, W. Kisel, W. M. Heath, J. Brett, D. M. Stern, *J. Biol. Chem.* **1994**, *269*, 25106 – 25119; b) J. Kao, Y. G. Fan, I. Haehnel, J. Brett, S. Greenberg, M. Clauss, M. Kayton, K. Houck, W. Kiesel, R. Seljelid, J. Burnier, D. M. Stern, *J. Biol. Chem.* **1994**, *269*, 9774 – 9782.

[7] T. A. Coleman, WO-A 96/40719.

[8] a) H. Schluesener, K. Seid, R. Meyermann, *Glia* **1997**, *20*, 365 – 372; b) H. Schluesener, K. Seid, R. Meyermann, *Acta Neuropathol.* **1999**, 97, 119 – 126; c) H. Wege, H. Schluesener, R. Meyermann, V. Barac-Latas, G. Suchanek, H. Lassmann, *Adv. Exp. Med. Biol.* **1998**, *440*, 437 – 444; U. E. Knies, H. A. Behrensdorf, C. A. Mitchell, U. Deutsch, W. Risau, H. C. Drexeler, M. Clauss, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12322 – 12327.

[9] a) M. P. Tas, J. C. Murray, *Int. J. Biochem. Cell. Biol.* **1996**, *28*, 837 – 841; b) M. R. Marvin, S. K. Libutti, M. Kayton, J. Kao, J. Hayward, T. Grikscheit, Y. Fan, J. Brett, A. Weinberg, R. Nowygrod, P. LoGerfo, C. Feind, K. S. Hansen, M. Schwartz, D. Stern, J. Chabot, *J. Surg. Res.* **1996**, *63*, 248 – 255; c) M. Schwartz, J. Brett, J. Li, J. Hayward, R. Schwarz, J. Kao, O. Chappey, J. L. Wautier, J. Chabot, P. LoGerfo, D. Stern, *Circulation* **1995**, *92* (Suppl.), 1 – 7.

[10] a) T. Boehm, J. Folkman, T. Browder, M. S. O'Reilly, *Nature* **1997**, *390*, 404 – 407; b) D. J. Falcone, K. M. Faisal Khan, T. Layne, L. Fernandes, *J. Biol. Chem.* **1998**, *273*, 31480 – 31485; c) L. Holmgren, M. S. O'Reilly, J. Folkman, *Nat. Med.* **1995**, *1*, 149 – 153; d) R. S. Kerbel, *Nature* **1997**, *390*, 335 – 336; e) M. S. O'Reilly, T. Boehm, Y. Shing, N. Fikai, G. Vasios, W. S. Lane, E. Flynn, J. R. Birkhead, B. R. Olsen, J. Folkman, *Cell* **1997**, *88*, 277 – 285.

  1433-7851/99/3824-3637 $ 17.50+.50/0