Volume 3 · Number 3 · 1993
GENE EXPRESSION

MINI REVIEW

Remarks on the mechanism of ribosome binding to eukaryotic mRNAs

Nahum Sonenberg

Department of Biochemistry and McGill Cancer Center, McGill University, Montreal, Quebec, Canada

Knowledge of the mechanism whereby ribosomes bind to mRNA is fundamental to the understanding of control of gene expression at the level of translation. In eukaryotes, numerous studies have shown that the major target of control of translation is at the ribosome binding step (Hershey, 1991). A large number of translation initiation factors (IFs, of which there are at least 10), in addition to ATP, participate in ribosome binding to mRNA in eukaryotes (for a recent review see Merrick, 1992). Such complexity is in stark contrast to the relative simplicity of this process in prokaryotes, where only three IFs, and no ATP hydrolysis, are required. In addition, all cellular (except organellar) mRNAs contain at their 5′ end a cap structure (m^7GpppN, where N is any nucleotide). These differences appear to be consistent with the view that the mechanisms by which ribosomes bind mRNA are fundamentally distinct between prokaryotes and eukaryotes (for example, see Kozak, 1983). In prokaryotes, the small 30S ribosomal subunit binds internally by a mechanism that involves base-pairing between the 16S rRNA and the Shine-Dalgarno sequence upstream of the initiator AUG (for a review see Gold et al., 1981). In eukaryotes, however, the small 40S ribosomal subunit is believed to gain access to the initiator AUG strictly via the 5′ end, in a mode that is facilitated by the cap structure (Kozak, 1989). It has been postulated further that following binding, the 40S ribosome reaches the initiator AUG by a mechanism termed "scanning," which requires that the ribosome moves vectorially in a 5′ to 3′ direction (Kozak, 1989). Notwithstanding these views, recent evidence has accumulated suggesting some significant similarities between prokaryotes and eukaryotes in the mechanisms of ribosome binding. In particular, some eukaryotic mRNAs initiate translation by internal ribosome binding, which displays features characteristic of translation in prokaryotes. This mini-review will describe these recent developments and offer some thoughts about possible modes by which ribosomes bind mRNA.

The "scanning" mechanism

The ribosome "scanning" model was initially proposed by Kozak and Shatkin (1977) to account for several important observations in eukaryotic translation that set it apart from prokaryotes. These include (a) the absence of a functional Shine-Dalgarno sequence or an analogous sequence upstream of the initiator AUG; (b) preference for initiating translation in eukaryotes at the first AUG; (c) most eukaryotic, as opposed to prokaryotic, mRNAs are monocistronic; and (d) the facilitating role of the cap in eukaryotic translation. The model states that the 40S ribosomal subunit binds at or near the 5′ end of the mRNA, in a manner that is facilitated, but not dependent, on the presence of the cap structure. This process was likened to threading through a needle's eye. The

Correspondence: Nahum Sonenberg, Department of Biochemistry, McGill University, 3655 Drummond Street, Montreal, PQ, Canada H3G 1Y6 Tel (514) 398-7274 Fax (514) 398-7384
© 1994 by Finch University of Health Sciences/The Chicago Medical School. All rights reserved. 1052-2166/93/0303/317-07$2.00

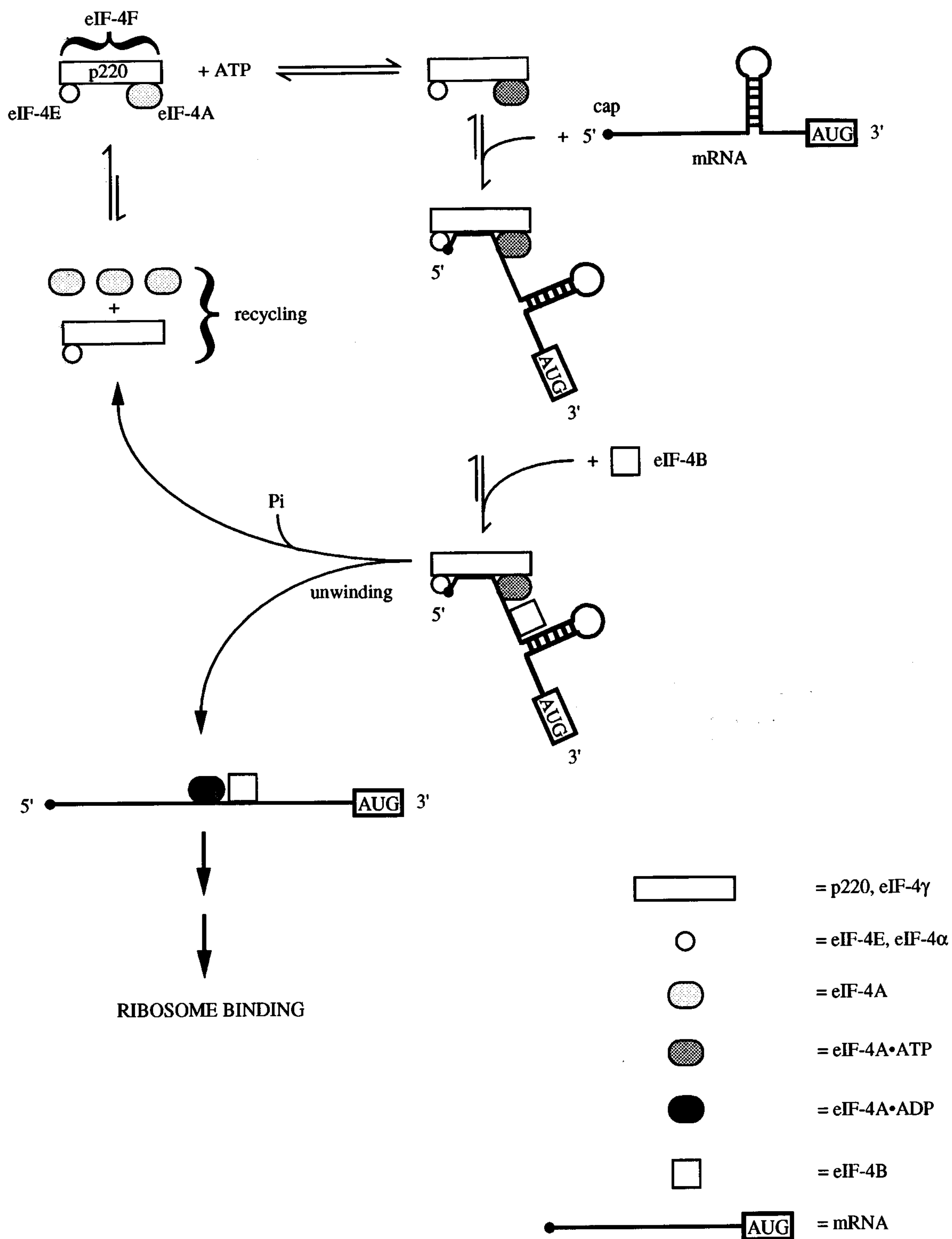

Figure 2. Model for the mechanism of action of mRNA binding initiation factors. The major steps are as follows: (1) Binding of eIF-4F to the cap structure of the mRNA, followed by the binding of eIF-4B. (2) Unwinding of the proximal 5′ secondary structure, in an ATP-dependent manner. (3) Recycling of eIF-4A through the eIF-4F complex. It is possible that recycling occurs on the mRNA. It is also possible that eIF-4F (perhaps the p220 subunit) signals ribosome association prior to its release from the mRNA. The figure is reproduced from Pause et al. (1994).

UTR; translation of all mRNAs is abolished in extracts prepared from yeast that are eIF-4A gene-disrupted (Altmann et al., 1990; Blum et al., 1992). Furthermore, dominant negative mutants of the mammalian eIF-4A inhibit translation of all mRNAs when added to translation extracts (Pause et al., 1994). Thus, it was suggested that eIF-4A is also involved in other helicase activities, such as melting of mRNA–rRNA interactions during translation initiation (Blum et al., 1992).

According to the model (Fig. 2), eIF-4F binds first to the mRNA cap structure. Binding affinity is largely determined by the interaction of the cap-binding subunit, eIF-4E, with the cap structure. This is determined by several factors, including the availability of the cap structure, the RNA secondary structure in the vicinity of the cap, and other undetermined factors (Rhoads, 1991; Thach, 1992). Binding of eIF-4F to the mRNA is also likely to be dependent on p220, as this subunit binds strongly to RNA, and eIF-4F binds more avidly to RNA than eIF-4E or eIF-4A by themselves (Jaramillo et al., 1991). Binding of eIF-4F to the mRNA is followed by the association of eIF-4B, and melting of secondary structure (Jaramillo et al., 1991). This melting generates a single-stranded RNA region that serves as a ribosome binding site. It is not clear from this model how much of the 5′ UTR has to be melted prior to ribosome binding. Also, the model does not address the question where and when the recycling of eIF-4A occurs—i.e., whether on the mRNA or in solution.

This model could also be applied to internal initiation, as its only new provision is that the cap and the 5′ end do not play a role in the ribosome attachment to a defined internal binding site. There are no general features that are common to all mRNAs that bind ribosomes internally. However, from work on picornaviruses, it is apparent that both primary and secondary, and probably tertiary, RNA structure play an important role in ribosome binding (Jackson et al., 1990; Jang et al., 1990; Meerovitch and Sonenberg, 1993). In addition, several trans-acting factors, which are not known to be required for translation of cellular mRNAs, appear to be required for efficient translation of picornavirus' mRNAs (Meerovitch and Sonenberg, 1993; McBratney et al., 1993). However, all of the general mRNA binding initiation factors—eIF-4A, eIF-4B, and eIF-4F—are likely to be required for internal initiation. The requirement for eIF-4A and eIF-4B has been known for some time (Staehelin et al., 1975), but a role for eIF-4F has been suggested only recently (Anthony and Merrick, 1991; Scheper et al., 1992; Pause et al., 1994). However, eIF-4F, which contains a cleaved p220 subunit, is inactive for cap-dependent translation (Etchison et al., 1982) but is apparently functional in cap-independent, internal ribosome binding. One possible role for the accessory factors in translation of picornavirus' RNA is to facilitate the binding of the translation factors to the RNA.

Similarities between eukaryotic and prokaryotic ribosome binding

One attractive feature of the model is its similarity in some important aspects to the mechanism of ribosome binding in prokaryotes, as in the latter the attachment of ribosomes to the mRNA requires a single-stranded RNA region (e.g., de Smit and van Duin, 1990). Furthermore, it has been shown that in prokaryotes, ribosomes can "scan" or diffuse bidirectionally along certain mRNAs following termination of translation, and reinitiate translation at the nearest downstream or upstream initiation codon (Adhin and van Duin, 1990). It was consequently suggested that both prokaryotic and eukaryotic ribosomes possess an inherent capacity to diffuse bidirectionally on the mRNA (Adhin and van Duin, 1990). This is consistent with observations that eukaryotic ribosomes can reinitiate translation upstream of a termination codon (Peabody and Berg, 1986; Thomas and Capecchi, 1986).

Another interesting analogy between the two systems was pointed out by Pilipenko et al. (1992). All picornaviruses contain in their 5′ UTR an oligopyrimidine sequence (first described for FMDV by Beck et al., 1983) positioned at a conserved distance (~20 nt) upstream of an AUG. This AUG is used as an initiator in some genera of the picornaviridae (for example, encephalomyocarditis virus), but not in other genera (for example, poliovirus; for reviews see Jackson et al., 1990; Jang et al., 1990; Meerovitch and Sonenberg, 1993). The oligopyrimidine sequence is required for ribosome binding (Kühn et al., 1990; Nicholson et al., 1991; Pestova et al., 1991), and so is the conserved spacing between the oligopyrimidine stretch and the AUG (Pilipenko et al., 1992).

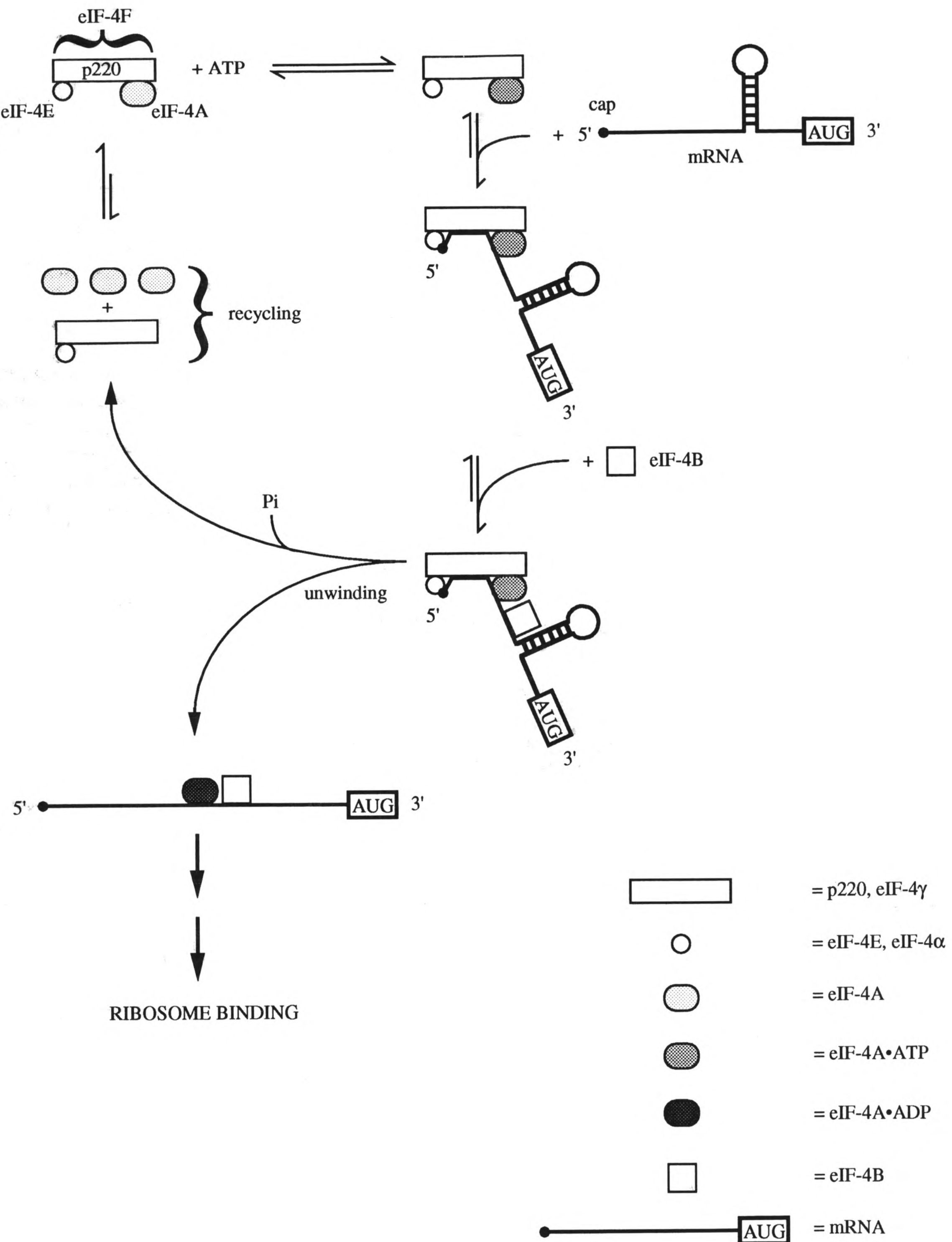

Figure 2. Model for the mechanism of action of mRNA binding initiation factors. The major steps are as follows: (1) Binding of eIF-4F to the cap structure of the mRNA, followed by the binding of eIF-4B. (2) Unwinding of the proximal 5′ secondary structure, in an ATP-dependent manner. (3) Recycling of eIF-4A through the eIF-4F complex. It is possible that recycling occurs on the mRNA. It is also possible that eIF-4F (perhaps the p220 subunit) signals ribosome association prior to its release from the mRNA. The figure is reproduced from Pause et al. (1994).

UTR; translation of all mRNAs is abolished in extracts prepared from yeast that are eIF-4A gene-disrupted (Altmann et al., 1990; Blum et al., 1992). Furthermore, dominant negative mutants of the mammalian eIF-4A inhibit translation of all mRNAs when added to translation extracts (Pause et al., 1994). Thus, it was suggested that eIF-4A is also involved in other helicase activities, such as melting of mRNA–rRNA interactions during translation initiation (Blum et al., 1992).

According to the model (Fig. 2), eIF-4F binds first to the mRNA cap structure. Binding affinity is largely determined by the interaction of the cap-binding subunit, eIF-4E, with the cap structure. This is determined by several factors, including the availability of the cap structure, the RNA secondary structure in the vicinity of the cap, and other undetermined factors (Rhoads, 1991; Thach, 1992). Binding of eIF-4F to the mRNA is also likely to be dependent on p220, as this subunit binds strongly to RNA, and eIF-4F binds more avidly to RNA than eIF-4E or eIF-4A by themselves (Jaramillo et al., 1991). Binding of eIF-4F to the mRNA is followed by the association of eIF-4B, and melting of secondary structure (Jaramillo et al., 1991). This melting generates a single-stranded RNA region that serves as a ribosome binding site. It is not clear from this model how much of the 5′ UTR has to be melted prior to ribosome binding. Also, the model does not address the question where and when the recycling of eIF-4A occurs—i.e., whether on the mRNA or in solution.

This model could also be applied to internal initiation, as its only new provision is that the cap and the 5′ end do not play a role in the ribosome attachment to a defined internal binding site. There are no general features that are common to all mRNAs that bind ribosomes internally. However, from work on picornaviruses, it is apparent that both primary and secondary, and probably tertiary, RNA structure play an important role in ribosome binding (Jackson et al., 1990; Jang et al., 1990; Meerovitch and Sonenberg, 1993). In addition, several trans-acting factors, which are not known to be required for translation of cellular mRNAs, appear to be required for efficient translation of picornavirus' mRNAs (Meerovitch and Sonenberg, 1993; McBratney et al., 1993). However, all of the general mRNA binding initiation factors—eIF-4A, eIF-4B, and eIF-4F—are likely to be required for internal initiation. The requirement for eIF-4A and eIF-4B has been known for some time (Staehelin et al., 1975), but a role for eIF-4F has been suggested only recently (Anthony and Merrick, 1991; Scheper et al., 1992; Pause et al., 1994). However, eIF-4F, which contains a cleaved p220 subunit, is inactive for cap-dependent translation (Etchison et al., 1982) but is apparently functional in cap-independent, internal ribosome binding. One possible role for the accessory factors in translation of picornavirus' RNA is to facilitate the binding of the translation factors to the RNA.

Similarities between eukaryotic and prokaryotic ribosome binding

One attractive feature of the model is its similarity in some important aspects to the mechanism of ribosome binding in prokaryotes, as in the latter the attachment of ribosomes to the mRNA requires a single-stranded RNA region (e.g., de Smit and van Duin, 1990). Furthermore, it has been shown that in prokaryotes, ribosomes can "scan" or diffuse bidirectionally along certain mRNAs following termination of translation, and reinitiate translation at the nearest downstream or upstream initiation codon (Adhin and van Duin, 1990). It was consequently suggested that both prokaryotic and eukaryotic ribosomes possess an inherent capacity to diffuse bidirectionally on the mRNA (Adhin and van Duin, 1990). This is consistent with observations that eukaryotic ribosomes can reinitiate translation upstream of a termination codon (Peabody and Berg, 1986; Thomas and Capecchi, 1986).

Another interesting analogy between the two systems was pointed out by Pilipenko et al. (1992). All picornaviruses contain in their 5′ UTR an oligopyrimidine sequence (first described for FMDV by Beck et al., 1983) positioned at a conserved distance (~20 nt) upstream of an AUG. This AUG is used as an initiator in some genera of the picornaviridae (for example, encephalomyocarditis virus), but not in other genera (for example, poliovirus; for reviews see Jackson et al., 1990; Jang et al., 1990; Meerovitch and Sonenberg, 1993). The oligopyrimidine sequence is required for ribosome binding (Kühn et al., 1990; Nicholson et al., 1991; Pestova et al., 1991), and so is the conserved spacing between the oligopyrimidine stretch and the AUG (Pilipenko et al., 1992).

This is very reminiscent of the arrangement of the Shine-Dalgarno and the initiator AUG in prokaryotes, and several potential regions in the 18S rRNA that can base-pair with the oligopyrimidine region have been noted (Nicholson et al., 1991; Pestova et al., 1991; Pilipenko et al., 1992).

Summary

It is evident from the data discussed here that the mechanism and the rules for mRNA binding in eukaryotes are complex and not well defined. The major points of this review are (1) ribosome binding could be preceded by the unwinding of mRNA secondary structure; (2) there is no obligatory ribosome entry through the 5′ end of the mRNA; (3) there is no obligatory linear "scanning" of the 5′UTR; and (4) there are some interesting similarities between prokaryotes and eukaryotes in the mode of ribosome binding to mRNA, particularly in the ability of the small ribosomal subunit to diffuse or "scan" on the mRNA, and in the requirement for a minimally structured RNA for efficient ribosome binding.

Acknowledgments

I thank M. Katze, T. Donahue, G. Belsham, A. Pause, T. Hohn, and O. Donze for comments on the manuscript.

The work from the author's laboratory was supported by a grant from the Medical Research Council of Canada.

References

R. D. Abramson, T. E. Dever, T. G. Lawson, B. K. Ray, R. E. Thach, and W. C. Merrick (1987), J Biol Chem 262, 3826–3832.

M. R. Adhin and J. van Duin (1990), J Mol Biol 213, 811–818.

M. Altmann, S. Blum, T. M. A. Wilson, and H. Trachsel (1990), Gene 91, 127–129.

M. Altmann, P. P. Muller, B. Wittmer, F. Ruchti, S. Lanker, and H. Trachsel (1993), EMBO J 12, 3997–4003.

D. D. Anthony and W. C. Merrick (1991), J Biol Chem 266, 10218–10226.

B. A. Arrick, A. L. Lee, R. L. Grendell, and R. Derynck (1991), Mol Cell Biol 11, 4306–4313.

E. Beck, S. Forss, K. Strebel, R. Cattaneo, and G. Feil (1983), Nucleic Acids Res 11, 7873–7885.

S. Blum, S. R. Schmid, A. Pause, P. Buser, P. Linder, N. Sonenberg, and H. Trachsel (1992), Proc Natl Acad Sci USA 89, 7664–7668.

A-M. Bonneau and N. Sonenberg (1987), J Biol Chem 262, 11134–11139.

L-J. Chang, P. Pryciak, D. Ganem, and H. E. Varmus (1989), Nature 337, 364–368.

A. M. Cigan, L. Feng, and T. F. Donahue (1988), Science 242, 93–97.

R. Coppolecchia, P. Buser, A. Stotz, and P. Linder (1993), EMBO J 12, 4005–4014.

J. Curran and D. Kolakofsky (1988), EMBO J 7, 2869–2874.

J. Curran and D. Kolakofsky (1989), EMBO J 8, 521–526.

A. Darveau, J. Pelletier, and N. Sonenberg (1985), Proc Natl Acad Sci USA 82, 2315–2319.

M. H. de Smit and J. van Duin (1990), Proc Natl Acad Sci USA 87, 7668–7672.

D. Etchison, S. C. Milburn, I. Edery, N. Sonenberg, and J. W. B. Hershey (1982), J Biol Chem 257, 14806–14810.

H. Fan and S. Penman (1970), J Mol Biol 50, 655–670.

N. Fouillot, S. Tlouzeau, J.-M. Rossignol, and O. Jean-Jean (1993), J Virol 67, 4886–4895.

J. Fütterer, Z. Kiss-Lazlo, and T. Hohn (1993), Cell 73, 789–802.

L. Gold, D. Pribnow, R. Schneider, S. Shinedling, B. W. Singer, and G. Stormo (1981), Annu Rev Microbiol 35, 365–403.

J. A. Grifo, R. D. Abramson, C. A. Satler, and W. C. Merrick (1984), J Biol Chem 259, 8648–8654.

C. Goyer, M. Altmann, H. S. Lee, A. Blanc, M. Deshmukh, J. L. Woolford, H. Trachsel, and N. Sonenberg (1993), Mol Cell Biol 13, 4860–4874.

S. Hambidge and P. Sarnow (1991), J Virol 65, 6312–6315.

J. W. B. Hershey (1991), Annu Rev Biochem 60, 717–757.

A. G. Hinnebusch (1990), Trends Biochem Sci 15, 148–152.

R. J. Jackson, M. T. Howell, and A. Kaminski (1990), Trends Biochem Sci 15, 477–483.

S. K. Jang, H.-G. Kräusslich, M. J. H. Nicklin, G. M. Duke, A. C. Palmenberg, and E. Wimmer (1988), J Virol 62, 2636–2643.

S. K. Jang, T. V. Pestova, C. U. T. Hellen, G. W. Witherell, and E. Wimmer (1990), Enzyme 44, 292–309.

R. Kühn, N. Luz, and E. Beck (1990), J Virol 64, 4625–4631.

A. E. Koromilas, A. Lazaris-Karatzas, and N. Sonenberg (1992), EMBO J 11, 4153–4158.

M. Kozak (1983), Microbiol Rev 47, 1–45.

M. Kozak (1989), J Cell Biol 108, 229–241.

M. Kozak and A. J. Shatkin (1977), J Mol Biol 112, 75–96.

D. G. Macejak and P. Sarnow (1991), Nature 353, 90–94.

S. McBratney, C. Y. Chen, and P. Sarnow (1993), Curr Opin Cell Biol 5, 961–965.
K. Meerovitch and N. Sonenberg (1993), Semin Virol 4, 217–227.
K. Meerovitch, Y. V. Svitkin, H. S. Lee, F. Lejbkowicz, D. J. Kenan, E. K. L. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg (1993), J Virol 67, 3798–3807.
W. C. Merrick (1992), Microbiol Rev 56, 291–315.
N. Methot, A. Pause, J. W. B. Hershey, and N. Sonenberg (1994), Mol Cell Biol, in press.
S. C. Milburn, J. W. B. Hershey, M. V. Davies, K. Kelleher, and R. J. Kaufman (1990), EMBO J 9, 2783–2790.
P. P. Mueller and A. G. Hinnebusch (1986), Cell 45, 201–207.
R. Nicholson, J. Pelletier, S-Y. Le, and N. Sonenberg (1991), J Virol 65, 5886–5894.
S.-K. Oh, M. P. Scott, and P. Sarnow (1992), Genes Dev 6, 1643–1653.
A. Pause, N. Methot, Y. Svitkin, W. C. Merrick, and N. Sonenberg (1994), EMBO J, in press.
D. S. Peabody and P. Berg (1986), Mol Cell Biol 6, 2704–2711.
J. Pelletier and N. Sonenberg (1988), Nature 334, 320–325.
J. Pelletier, G. Kaplan, V. R. Racaniello, and N. Sonenberg (1988), Mol. Cell Biol 8, 1103–1112.
T. V. Pestova, C. U. T. Hellen, and E. Wimmer (1991), J Virol 65, 6194–6204.
E. V. Pilipenko, A. P. Gmyl, S. V. Maslova, Y. V. Svitkin, A. N. Sinyakov, and V. I. Agol (1992), Cell 68, 119–131.
B. K. Ray, T. G. Lawson, J. C. Kramer, M. H. Cladaras, J. A. Grifo, R. D. Abramson, W. C. Merrick, and R. E. Thach (1985), J Biol Chem 260, 7651–7658.
C. D. Rao, M. Pech, K. C. Robbins, and S. A. Aaronson (1988), Mol Cell Biol 8, 284–292.
R. E. Rhoads (1991), in Translation in Eukaryotes (H. Trachsel, ed.), CRC Press, Boca Raton, FL, pp. 109–148.
F. Rozen, I. Edery, K. Meerovitch, T. E. Dever, W. C. Merrick, and N. Sonenberg (1990), Mol Cell Biol 10, 1134–1144.
G. C. Scheper, H. O. Voorma, and A. A. M. Thomas (1992), J Biol Chem 267, 7269–7274.
N. Sonenberg, M. A. Morgan, W. C. Merrick, and A. J. Shatkin (1978), Proc Natl Acad Sci USA 75, 4843–4847.
N. Sonenberg, D. Guertin, and K. A. W. Lee (1982), Mol Cell Biol 2, 1633–1638.
N. Sonenberg (1988), Prog Nucleic Acid Res Mol Biol 35, 173–207.
N. Sonenberg (1991), Trends Genet 7, 105–106.
T. Staehelin, H. Trachsel, B. Erni, A. Boschetti, and M. H. Schreier (1975), FEBS Proc Meet, pp. 309–323.
V. L. Stroehrer, E. M. Jorgensen, and R. L. Garber (1986), Mol Cell Biol 6, 4667–4675.
T. E. Thach (1992), Cell 68, 177–180.
A. A. M. Thomas, G. C. Scheper, and H. O. Voorma (1992), New Biol 4, 404–407.
K. R. Thomas and M. R. Capecchi (1986), Nature 324, 34–38.
K. Tsukiyama-Kohara, N. Iizuka, M. Kohara, and A. Nomoto (1992), J Virol 66, 1476–1483.
R. Yan, W. Rychlik, D. Etchison, and R. E. Rhoads (1992), J Biol Chem 267, 23226–23231.