Annals of Arid Zone 36(2): 129-132, 1997

Polysomal and Post-polysomal RNA in Developing Barley Embryos and their Possible Role as Conserved Messages During Germination

V.K. Yadav
Department of Biochemistry, SKN College of Agriculture, Jobner 303 329, India

Abstract: Sharp increase in post-polysomal RNA, accompanied by decrease in polysomal RNA from 24 days after anthesis to mature embryos, has indicated presence of conserved messages in mature embryos. Majority of these conserved messages were translated during 0 to 6 h germination stage, and a few others during 30 to 36 h stage. Both polysomal and post-polysomal RNA were more in Notch-2 than in NP 113, but conserved messages were translated to a greater extent in NP 113.

Key words: Polysomal RNA, post-polysomal RNA, conserved messages, *Hordeum vulgare.*

The improvement in nutritional quality of high lysine barley mutant, Notch-2, compared to its parent, NP 113, appears to be due to reduction in synthesis of lysine-poor storage proteins, hordein (Bansal *et al.*, 1977). The endosperms of Notch-2 have lesser number of hordein polypeptides as compared to the parent. Regulation at the level of transcription/post-transcription may be responsible for lower accumulation of hordein in the mutant endosperms (Bhattacharya *et al.*, 1986a; Tyagi *et al.*, 1992).

Studies on embryos of NP 113 and Notch-2 grains have shown that although they transcribe hordein mRNA (Bhattacharya *et al.*, 1986b), hordein polypeptides are not synthesized in them. The absence of hordein polypeptides in embryos is possibly due to formation of ribonucleoprotein complexes (Yadav *et al.*, 1990). But, the information on pattern of RNA synthesis in these embryos during seed development is lacking. Present study was, therefore, undertaken to analyze polysomal and post-polysomal RNA in developing embryos and their possible role as conserved messages during germination.

Material and Methods

Barley (*Hordeum vulgare* L.) seed of NP 113 and Notch-2 were collected at 17 and 24 days after anthesis (DAA) and at maturity from the field-grown crop. Embryos from these seeds were taken out manually and polysomal and post-polysomal RNA were isolated as described earlier (Yadav, 1993). For germination study, dry seeds were surface-sterilized with 0.02% $HgCl_2$ for 5 minutes. After several washings with sterile distilled water, the seeds were imbibed for 30 minutes and embryos were taken out. *In vivo* labeling of proteins during germination of these embryos was carried out as described by Sanchez and Aguilar (1984) using (^{14}C) chlorella protein hy-

drolyzate (specific activity 26 mci m^{-1} atoms) in presence or absence of α-amanitin (10 μg ml^{-1}). The pulse was given for 6 h each at two germination stages, viz., 0 to 6 and 30 to 36 h. On completion of incubation time, polysomal pellet was obtained by centrifugation at 1,05,000 g for 90 minutes and post-polysomal pellet at 2,50,000 g for 5 h, using SW 65 rotor in Bechman L2-65B ultra centrifuge, as described earlier (Luthe and Peterson, 1977; Nichols and Welder, 1983). These pellets were dissolved in 0.1 M phosphate buffer (pH 7.5) and proteins were precipitated using 10% TCA, which were termed as polysomal proteins and post-polysomal proteins, respectively. Soluble proteins were recovered from the supernatant (obtained after sedimenting post-polysomal pellet at 2,50,000 g) by precipitation with 9 volumes of chilled acetone at -20°C for 2 h. Radioactive counts in these proteins were measured in LKB liquid scintillation counter. RNA isolated from polysomal and post-polysomal pellets were termed as polysomal RNA (which includes rRNA and active mRNA) and post-polysomal RNA (which includes tRNA and conserved mRNA), respectively. For isolating RNA, the pellets were dissolved in 100 mM sodium glycerate buffer (pH 8.5) containing 100 mM EDTA, 100 mM NaCl, 0.05 mg ml^{-1} proteinase K and 0.05% SDS. The mixture was extracted with phenol-chloroform and RNA precipitated in aqueous phase with 2.5 volume of ethanol at -20°C overnight.

Results and Discussion

Content of polysomal RNA was more than post-polysomal RNA in developing embryos of both NP 113 and Notch-2 at 17 and 24 DAA, while the trend was reverse at maturity (Table 1). Post-polysomal RNA accounted for 44% of the total RNA at 17 DAA and 43% at 24 DAA. In mature embryos, fractions of post-polysomal RNA were 80% and 88%, respectively, in NP 113 and Notch-2. This suggests that towards maturity, most of RNA becomes repressed (conserved) in post-polysomal fraction. This is supported by our earlier study (Yadav *et al.*, 1990) which has shown presence

Table 1. RNA contents in developing embryos of NP 113 and Notch-2 barley grains

Developmental stage	Variety	Weight of 100 embryos (g)	Polysomal RNA (μg/100 embryos)	Post-polysomal RNA (μg/100 embryos)
17 DAA	NP 113	0.18	84 (466)	66 (367)
	Notch-2	0.19	95 (500)	74 (389)
24 DAA	NP 113	0.35	190 (543)	143 (409)
	Notch-2	0.40	242 (605)	183 (458)
Maturity	NP 113	0.37	72 (195)	289 (781)
	Notch-2	0.40	93 (221)	546 (133)

DAA = Days after anthesis.
The results are average of three replicates.
The values in parenthesis are μg RNA per g embryo.

Table 2. Incorporation of (^{14}C) amino acids into proteins from germinating embryos of NP 113 and Notch-2 barley in presence and absence of ∝-amanitin

Germination stage (h)	Variety	Cpm per mg embryo								
		Polysomal proteins			Post-polysomal proteins			Soluble proteins		
		Without ∝-amanitin	With ∝-amanitin	% inhibition	Without ∝-amanitin	With ∝-amanitin	% inhibition	Without ∝-amanitin	With ∝-amanitin	% inhibition
0-6	NP 113	2550	1094	26	3420	2425	29	5006	4012	20
	Notch-2	1990	1420	29	3670	2221	40	3892	3007	23
30-36	NP 113	2737	1250	54	3372	1928	43	8915	2840	68
	Notch-2	2795	1025	63	2386	1102	54	2947	525	77

Embryos were excised from NP 113 and Notch-2 barley grains and allowed to germinate in absence or presence of ∝-amanitin; (^{14}C) chlorella hydrolysate was added at 0 and 30 h germination stage and incubation was carried for 6 h in each case. Chloramphenicol was added to prevent bacterial contamination. The data are average of three replicates.

of ribonucleoprotein complexes in these barley embryos. Further, synthesis of majority of proteins by conserved messages at 0 to 6 h germination stage (Table 2) also supports this view. The results also showed that both polysomal and post-polysomal RNA were more in Notch-2 embryos than in NP 113 at all the stages at which study was conducted (Table 1).

Presence of α-amanitin (10 μg ml^{-1}) in incubation medium during germination of NP 113 and Notch-2 embryos inhibited the incorporation of amino acids in all the three protein fractions at the two studied germination stages (Table 2). The maximum inhibition was shown by soluble proteins at 30 to 36 h stage. The inhibitions at this stage were 68% and 77% of control (without α-amanitin) in NP 113 and Notch-2, respectively. The results showed that incorporation of amino acids into different protein fractions was inhibited to a higher extent in Notch-2 embryos than NP 113.

Synthesis of new mRNA is inhibited by α-amanitin. This implies that lower the inhibition in amino acids incorporation in presence of α-amanitin, higher is the contribution of conserved messages towards protein synthesis. The inhibition pattern of amino acid incorporation due to α-amanitin, therefore, indicates that at early stage of germination (0 to 6 h), majority of proteins belong to conserved mRNA in both the varieties. The situation is reverse at the later stage (30 to 36 h) when most proteins are synthesized from newly formed mRNA, but to a greater extent in Notch-2 than in NP 113. A selective mechanism (Sanchez and Aguilar, 1984) appears to be operative particularly in NP 113 embryos, which modulates translation of conserved transcripts so that majority are translated during early stage, while few others are translated only at later stage of germination. However, possibility of differential uptake of α-amanitin in NP 113 and Notch-2, leading to

its different effect in blocking mRNA in the two cases, can not be ruled out.

Acknowledgement

Author is thankful to the Director, IARI, New Delhi, for providing fellowship during the tenure of this work.

References

Bansal, H.C., Srivastava, K.N., Eggum, B.O. and Mehta, S.L. 1977. Nutritional evaluation of high protein genotypes of barley. *Journal of Science, Food and Agriculture* 29: 157-160.

Bhattacharya, K., Mehta, S.L. and Banerjee, D. 1986a. Serological characterization of hordein from *Hordeum vulgare*. *Indian Journal of Experimental Biology* 24: 445-448.

Bhattacharya, K., Mehta, S.L. and Chandra, G.R. 1986b. Tissue specific translational control of hordein biosynthesis in barley grains. *Indian Journal of Experimental Biology* 24: 599-604.

Luthe, D.S. and Peterson, D.M. 1977. Cell free synthesis of globulin by developing oat (*Avena sativa*) seeds. *Plant Physiology* 59: 836-841.

Nichols, J.L. and Welder, L. 1983. The proteins in free cytoplasmic poly (A^+) ribonucleoprotein complex from maize. *Plant Science Letters* 29: 61-66.

Sanchez, D.J.E. and Aguilar, R. 1984. Protein synthesis patterns: Relevance of old and new messenger RNA in germinating maize embryos. *Plant Physiology* 75: 231-234.

Tyagi, A., Santha, I.M. and Mehta, S.L. 1992. Regulation of hordein synthesis in barley high lysine mutant Notch-2. *Indian Journal of Biochemistry and Biophysics* 29: 93-96.

Yadav, V.K. 1993. Polysomes and RNA analysis of high lysine barley mutant Notch-2 and its parent NP 113. *Annals of Arid Zone* 32: 201-202.

Yadav, V.K., Santha, I.M. and Mehta, S.L. 1990. Characterization of free cytoplasmic poly (A^+) ribonucleoprotein complexes from barley embryos. *Indian Journal of Experimental Biology 28: 268-272.*