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Detection of a sub-set of polysomal mRNAs associated with modulation of hypusine formation at the G1-S boundary† Proposal of a role for eIF-5A in onset of DNA replication

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Abstract S phase entry, i.e. start of DNA replication, is a crucial step in proliferation. Inhibition of S phase entry correlates with inhibition of hypusine formation, an event affecting only the eukaryotic initiation factor 5A (eIF-5A). Its hypusine-containing sequence was postulated to authorize polysomal utilization of specific transcripts for proteins necessary to enable DNA replication. Using mimosine to reversibly suppress the hypusine-forming deoxyhypusyl hydroxylase (E.C. 1.14.99.29) in cells while differentially displaying their polysomal versus non-polysomal mRNA populations, we report the detection and classification of several mRNA species that indeed disappear from and reappear at polysomes in concert with inhibition and disinhibition, respectively, of hypusine formation. Based on initial sequence data, two translationally controlled enzymes, both critical for proliferation, are identified as candicate products of such mRNAs, methionine adenosyltransferase (E.C. 2.5.1.6) and cytochrome-*c* oxidase (EC 1.9.3.1) subunit I. The existence of such putative hypusine-dependent messenger nucleic acids (*hymns*) provides the basis for a proposal on their molecular function in onset of multiplication.

Key words: Post-translational modification; eIF-5A; Hypusine; Cell cycle control; Translational control; mRNA

1. Introduction

The unique amino acid hypusine [N^{ε}-(4-amino-2(*R*)-hydroxybutyl)-L-lysine] is formed post-translationally by the sequential action of deoxyhypusyl synthase (DOHS) and deoxyhypusyl hydroxylase (DOHH) on a single lysyl residue in only one cellular protein, the eukaryotic initiation factor 5A (eIF-5A) [1–3]. The hypusine-containing domain shows remarkable interspecies conservation, and a sequence of 12 amino acids containing the hypusine residue has been strictly conserved throughout eukaryotic evolution, from fungi to plants, insects, and mammals [3]. Hypusine formation, which has not been demonstrated in prokaryotes [2], is an absolute requirement for growth of *S. cerevesiae* [4], and a number of reports have established correlations between hypusine formation and the proliferation of eukaryotic cells (see [2,3]). However, the exact physiological function of this presumed translation initiation factor is unknown, and at present it even is unclear whether eIF-5A functions at the pretranslational and/or the translational level [3].

Deoxyhypusyl hydroxylase (E.C. 1.14.99.29), which catalyses the final step of hypusine biosynthesis, is apparently related [5,6] to other protein hydroxylating enzymes such as prolyl 4-hydroxylase (E.C 1.14.11.2) [7] and is effectively inhibited by mimosine (3-(*N*-(3-hydroxypyridin-4-one))-2[*S*]-aminopropionic acid), both in vitro and in culture [3]. This coincides with proliferative arrest in the late G1 phase of the cell cycle [3,8–11]. Fast recovery of cellular DOHH activity occurs upon mimosine removal, an effect paralleled by passage of the G1-S boundary; in CHO cells, within 120 min hypusine levels recover to 50% of controls and S phase is entered in a highly synchronous manner [3]. Mimosine blocks S phase entry by interfering with the initiation of DNA replication, and its removal is followed by rapid re-initiation of replication; in LAZ463 cells, bromodeoxyuridine incorporation increases as early as 30 min after deblocking, when conventional flow cytometry (CFC) does not yet detect S phase traverse [9]. After 60 min in inhibitor-free medium, with CFC still not revealing resumption of S phase entry, the levels of the proliferation-related mRNAs for phosphoprotein p53 and histone H4 already are changing in accord with their physiological alterations during G1-to-S transit [3]. Re-addition of mimosine at that time no longer blocks G1-to-S passage in HL60 [8] or CHO cells (H.M.H-A., M. Lalande, unpublished observation), and S phase entry and further cell cycle progression continue in the presence of previously antiproliferative levels of mimosine [8].

These studies led to the proposal that the highly conserved hypusine-containing domain of eIF-5A would act as a selector for a class of specific mRNA substrates, the polysomal utilization of which then provides the tools to start up the DNA replication machinery [3]. If this is the case, it follows that mRNA species exist which disappear and reappear at polysomes in concert with inhibition and disinhibition, respectively, of cellular hypusine formation. Therefore, we have compared the differentially displayed patterns of polysomal mRNAs isolated from control cells; from cells exposed to concentrations of mimosine achieving both DOHH suppression

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and block in late G1 phase; and from cells released from mimosine arrest for a just short, cytokinetically defined period.

We report the detection and classification of a sub-set of polysomal mRNAs termed putative hypusine-dependent messenger nucleic acids (*hymns*) as they are associated with the mimosine-mediated modulation of hypusine formation and of G1-S transit. We identify two candidate *hymn*-related proteins and provide a conceptual framework for the mode of action of *hymns*.

2. Materials and Methods

2.1. Materials

Mimosine was obtained from Aldrich, Milwaukee, WI. All chemicals and reagents were of the highest quality commercially available. Human lymphoblastoid IM-9 cells were from American Type Tissue Culture, Bethesda, MD.

2.2. Cell culture and viability testing

IM-9 cells were maintained in RPMI-1640 supplemented with 10% fetal calf serum and 2 mM glutamine in an atmosphere of 5% CO_2/95% air at 37°C at a density of about 3×10^5 cells/ml. Viability was assessed by trypan blue exclusion and release of cytoplasmic LDH into the supernatant [6].

2.3. Determination of deoxyhypusine and hypusine

Logarithmically proliferating IM-9 cells (500,000/ml), in the absence or presence of 400 μM mimosine, were labeled for 18 h with 5 μCi/ml [*terminal methylenes*-^{3}H]spermidine (31 Ci/mmol; New England Nuclear). Control and inhibited cells were immediately harvested [3]; the subset of the mimosine-exposed cells used to assess reversal of DOHH inhibition, was transferred into fresh, isotope- and inhibitor-free medium for 30 min, and then processed [3].

2.4. Flow cytometry

At the indicated times, samples containing 200,000 cells/ml were stained with acridine orange as described [12] and immediately analyzed on an Coulter Epics flow cytometer.

2.5. Purification of mRNAs

Control, mimosine-blocked and -deblocked IM-9 cells were washed twice in phosphate-buffered saline and re-centrifuged. The cell pellet was resuspended in 2 ml buffer (10 mM Tris-HCl, pH 7.4; 0.15 M KCl; 4 mM $MgCl_2$; 0.0005% Triton X-100) and lysed with a dounce homogenizer. Nuclei were pelleted (2,000 rpm, 5 min), followed by centrifugation to remove mitochondria (10,000 rpm, 15 min; Sorval SS34). The post-mitochondrial supernatant was adjusted to 0.1% Triton X-100 and incubated at 4°C for 30 min to release membrane-bound polysomes. Polysomes, monosomes, and free RNA of each sample were then separated by centrifugation on a linear 15–50% sucrose gradient in 30 mM Tris-HCl, pH 7.0, 100 mM NaCl, 10 mM $MgCl_2$ (28,000 rpm, 4°C, 4 h; Beckman SW40.1). 0.7 ml fractions of this gradient were collected and the separation pattern monitored at 254 nm. Fractions representing the three peaks obtained were pooled and concentrated by precipitation using two volumes of ethanol. After 12 h at −20°C, the precipitate of each pool was harvested by centrifugation (10,000 rpm, 4°C, 15 min; Sorval SS34) and resuspended in 4 M guanidinium isothiocyanate. The total RNA obtained per peak was then isolated by the cesium chloride step-gradient method [13]. The purified polysomal, ribosomal, or free RNA of each sample was dissolved in diethyl pyrocarbonate-treated water, quantified, and equal quantities applied in the differential display assay.

2.6. Differential display of mRNA samples

To simultaneously visualize the mRNAs of relevant samples, the RNAmap kit (GenHunter, Boston, MA) was used. This technique involves (i) reverse transcription with oligo-dT primers anchored to the start of the poly(A)tails of the mRNAs in the sample; (ii) subsequent PCR amplification in the presence of a second oligonucleotide of arbitrary sequence; and (iii) electrophoretic separation of the [α-^{35}S]dATP-labeled cDNA products on a 6% polyacrylamide gel under denaturing conditions [14,15].

2.7. Cloning, sequencing, and identification of differentially displayed bands

Bands of interest were reamplified, using the primer sets and PCR conditions specified for the RNAmap kit. These PCR products were immediately cloned employing the pCR-TRAP) cloning system (GenHunter, Boston, MA). The MAX Efficiency DH10B) strain of competent *E. coli* (Gibco, Gaithersburg, MD) was used for transformation. Plasmids were prepared by boiling method and sequenced using the Sequenase Version 2.0 kit (Amersham-USB, Cleveland, OH). Results were referenced against the non-redundant nucleotide sequence databases of the National Center for Biotechnology Information (NCBI) using the BLAST program.

3. Results and discussion

In investigating the postulated existence of mRNA species that depend on eIF-5A for selective translation of proteins gating S-phase entry and onset of DNA replication [3], we defined three operational criteria to guide the experimental identification of such candidate transcripts. Biochemically, **Criterion I** requires that in cells subjected to complete suppression of hypusine formation, certain mRNAs selectively disappear from the polysomes. **Criterion II** specifies that these mRNAs, denied polysomal localization in parallel with inhibition of DOHH, relocate in (i) both the monosomal and free mRNA pools [IIa]; (ii) in only the monosomal [IIb] or the free [IIc] mRNA pool; or (iii) they completely disappear [IId]. In each case, however, **Criterion III** demands that such mRNAs reoccur at the polysomes of cells within a critical, brief period *after* their DOHH is reactivated and *before* G1-to-S transit resumes. The disappearance (Criterion I) and re-appearance (Criterion III) of polysomal mRNAs coincident with suppression and resumption of DOHH activity, respectively, is used to define these transcripts as putative hypusine-dependent messenger nucleic acids (*hymns*). Criterion II is only used for their categorization. Cytokinetically, Criterion I requires demonstration of complete arrest in late G1, Criterion III is satisfied by evidence for imminent exit from G1 not yet detectable by CFC. All three criteria must be met independent of the technique used for inhibition of cellular hypusine formation. Differential splicing could cause assignment of the same putative *hymn* to more than one class.

By definition, Class IIa-d putative *hymns* are translationally expressed in a manner dependent on hypusine synthesis, rather than on transcription. In every other regard, however, these transcripts must be very diverse; for example, IId *hymns* by definition are instable and may display rapid degradation motifs, whereas IIa *hymns* by definition are stable, but translationally masked. IIb and IIc *hymns* also are defined as translationally masked though present in monosome or free form, respectively. It is significant to caution that Criteria I–III are definitions, i.e. not contaminated by any concern for the actual biological function of the eventual protein products. Such function, however, may very well impact on the identification process applying Criteria I–III. If, for example, one of these protein products should itself act by facilitating the translation of other mRNAs, which a priori cannot be ruled out, then Criteria I–III will rank these other mRNAs among the putative *hymn* transcripts, although their dependency on hypusine formation is indirect only. Irrespective of whether Criteria I–III-defined transcripts are directly or indirectly dependent on hypusine formation, identification of each putative *hymn* must reveal translational regulation of its expression, and its enabling role

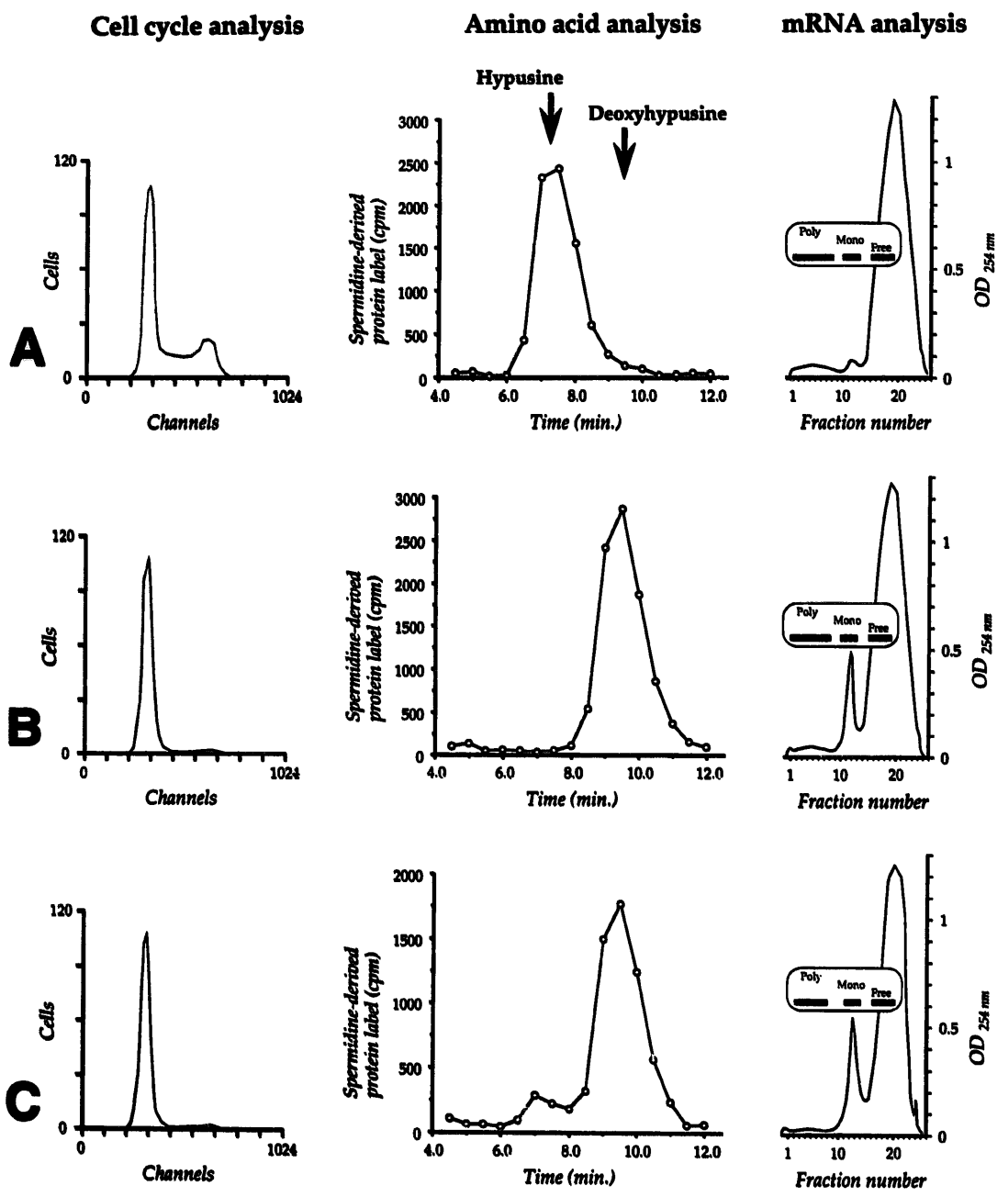

Fig. 1. Simultaneous determination in control (panel A), mimosine-blocked (panel B), and 30 min de-blocked (panel C) IM-9 cells of: (i) cell cycle distribution; (ii) hypusine-to-deoxyhypusine conversion; and (iii) the polysomal, monosomal, and free mRNA pools.

in multiplication. For classification as definitive *hymn*, additional experiments need to demonstrate that each putative *hymn* meets these essentials: (i) direct interaction with eIF-5A; (ii) DOHH-dependent translation of its encoded protein; and (iii) activity of this encoded protein in S phase inlet events. Therefore, Criteria I–III are necessary, but not sufficient to establish the directly hypusine-gated events in translation and in G1 exit.

The chemicals currently available for reversible inhibition of hypusine synthesis, and thus for applying Criteria I–III, may have multiple, mechanistically-unrelated, confounding effects on the complex cascade of cell cycle progression. Among these agents, only mimosine has been investigated in sufficient enzymological and cytokinetic detail to be used with confidence in experiments probing Criteria I–III. Mimosine and mimosine-like compounds directly interact with the active site of DOHH in vitro [3,5,6]. Complete suppression of cellular hypusine formation, without affecting biosynthesis of the peptidyl deoxyhypusine precursor and without overt cytotoxicity, is achieved within 16–18 h after addition of 200–500 μM mimosine, depending on cell type, and this effect coincides with arrest of exit from the G1 phase [3,8–11]. After mimosine removal, the

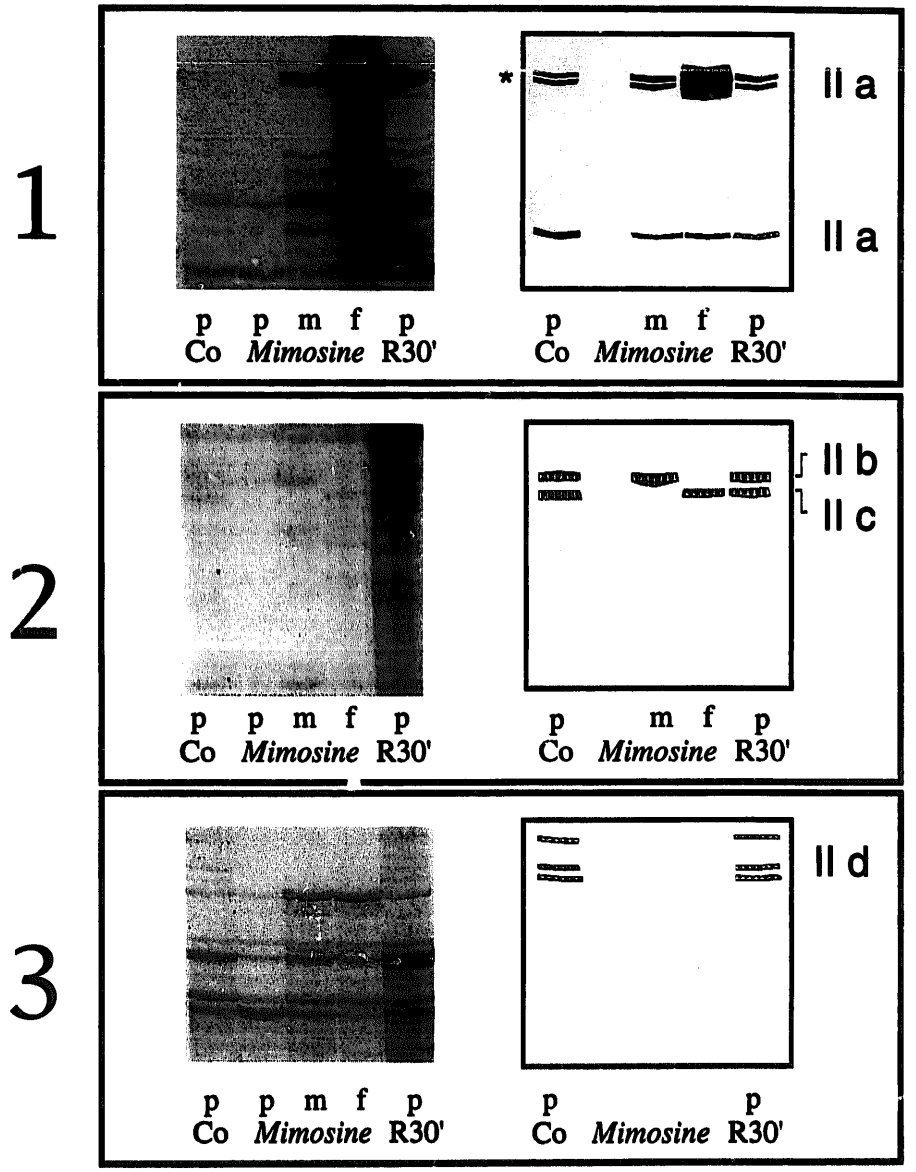

Fig. 2. Differential display of purified mRNA pools outlined in Fig. 1. A 5′-T_{12}MC-3′ primer anchored to the 5′-boundary of the poly(A) tails was used for reverse transcription, followed by PCR amplification with a second 10-mer of arbitrary sequence (Panel 1, 5′-AGGTGACCGT-3′; panel 2, 5′-GACCGCTTGT-3′; panel 3, 5′-GGTACTCCAC-3′) [14,15]. The asterisk indicated the band segment that was reamplified, cloned, and sequenced in an effort to gain information on translationability. **Left**, autoradiographic bands of [α-^{35}S]dATP-labeled cDNAs obtained from the following mRNA pools: polysomal, of control cells (p Co); polysomal/monosomal/free, of cells incubated with mimosine, to give complete inhibition of the hypusine-forming enzyme deoxyhypusyl hydroxylase, DOHH (p/m/f *Mimosine*); and polysomal, of cells released for 30 min from mimosine-mediated deoxyhypusyl hydroxylase inhibition, to allow for recovery of enzyme activity (p R30′). **Right**, schematic highlight of each autoradiography identifying Criterion I (transcript disappearance from polysomes upon DOHH inhibition) and Criterion III (transcript reappearance at polysomes upon DOHH disinhibition), and classifying each band according to Criterion IIa–IId (transcript distribution upon DOHH inhibition).

deoxyhypusine-to-hypusine conversion swiftly resumes [3], and within less than 1 h re-initiation of DNA replication becomes detectable [9], the deblocked cells thereafter rapidly progressing into S phase in a synchronized manner [3,8–11] and returning to asynchronous proliferation in later cell cycles (H.M.H-A., L.S.-C, unpublished observation). Several subsequent reports confirmed the suppressive effect of mimosine on G1 exit [16–22] and on the initiation of DNA replication [23–26], and verified reversibility of these effects upon mimosine removal [22,24,25]. Consequently, we added mimosine to logarithmically growing cultures of IM-9 cells to inhibit DOHH until arrest at the G1-S boundary occurred, and then briefly released the cells from the mimosine block by transfer to fresh medium. Inhibited, released, and control samples were monitored for viability, cell cycle distribution and hypusine formation. Viability of the mimosine-exposed populations did not differ from controls, as assessed by Trypan blue exclusion and release of cytoplasmic LDH (data not shown). Each population was used to isolate by sucrose gradient centrifugation a polysomal, a monosomal, and a free mRNA pool. The results are shown in Fig. 1. When

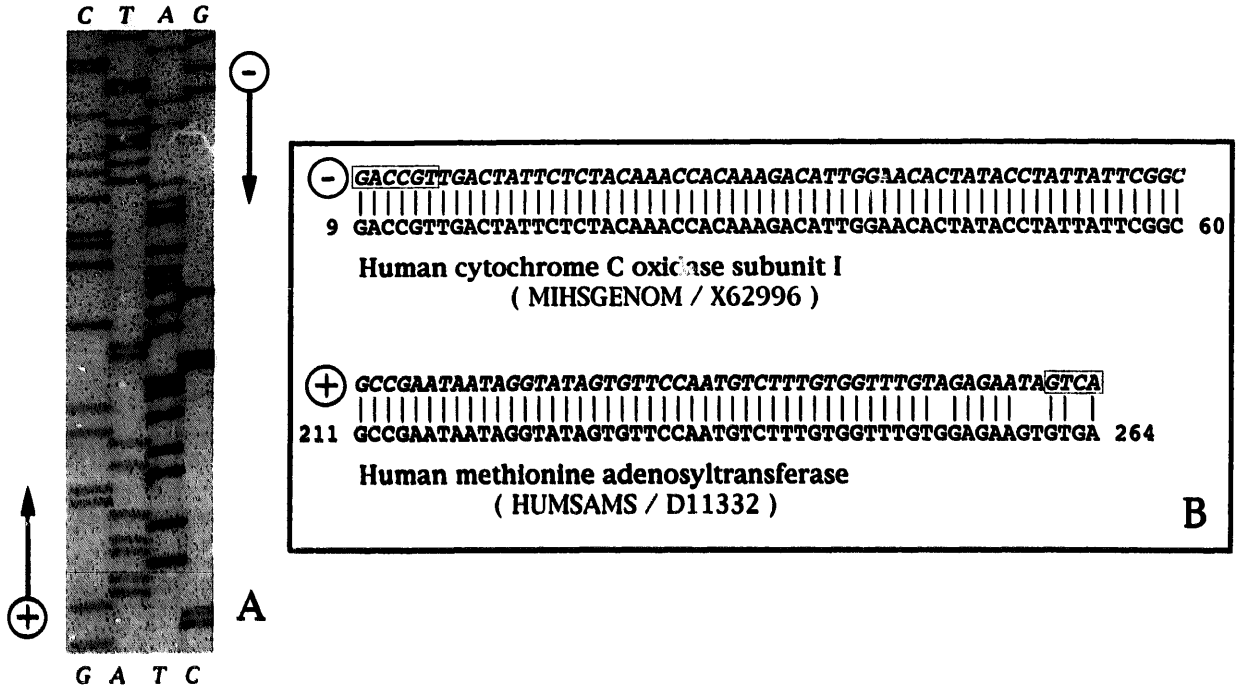

Fig. 3. Analysis result of the Class IIA putative *hymn* asterisk-marked in Fig. 2. The sequencing gel of the cloned insert is shown in (A). Strand and counterstrand reading directions are indicated. The two high-scoring segment pairs identifying cytochrome-*c* oxidase subunit I and methionine adenosyltransferase are identified together with their Accession Numbers (B). The boxed letters indicate arbitrary primer sequence.

compared to asynchronously logarithmically proliferating controls (Panel A), exposure to 400 μM mimosine for 16 h (Panel B) caused complete arrest of both transit of the G1-S boundary and of hypusine formation, the latter resulting in accumulation of deoxyhypusyl precursor. A marked increase in the relative size of the monosomal mRNA pool was also observed. This is interpreted to reflect a translationally quiescent, yet readied status of the reversibly nondividing population. 30 min after removal of mimosine by transferring the cells into fresh medium (Panel C), this prominence of the monosomal mRNA pool persisted, and the cells were still arrested in G1 and had not yet entered S phase, as documented by CFC. DOHH activity, however, had resumed, and the deoxyhypusine-to-hypusine conversion had progressed to 12% of the controls. Conversion continued to increase, reaching 40% after 150 min when S phase entry became evident by CFC (data not shown; [3]). The 30 min-release time point (R30′) was used for analysis of polysomal mRNAs, displayed side-by-side with samples from control and mimosine-inhibited cells.

Representative findings obtained by differential display [14,15,27] of these distinct subcellular mRNA populations are shown in Fig. 2, a novel application for this technique which until now has been used exclusively on total cellular mRNA to identify differentially expressed genes (e.g. [27–32]). The three panels show autoradiograms of [α-^{35}S]dATP-labeled cDNAs obtained from the following mRNA pools: polysomal, of control cells (p Co); polysomal/monosomal/free, of inhibited cells (p/m/f *Mimosine*); and polysomal, of deblocked cells (p R30′). After purification of the particular mRNAs from the appropriate cells, reverse transcription was achieved with a 5′-T_{12}MC-3′ primer anchored to the 5′-boundary of the poly(A) tails. The products were then subjected to PCR amplification, using a second 10-mer of arbitrary sequence. For panel 1, this second 10-mer was 5′-AGGTGACCGT-3′; for panel 2, 5′-GACCGCTTGT-3′; and for panel 3, 5′-GGTACTCCAC-3′. The cDNA subpopulations obtained, representing the 3′ termini in the indicated mRNA pools, were distributed on a DNA sequencing gel under denaturing conditions, and visualized. Clearly, the autoradiographs of panels 1, 2, and 3 show numerous bands for the polysomes of control cells, the polysomes of mimosine-inhibited cells, and the polysomes of cells deblocked for 30 min. Mimosine exposure, i.e. DOHH inhibition, for 16 h resulted in the loss of only a few polysomal bands relative to control polysomes (Criterion I), but did not affect the presence of these bands in the monosomal and/or free mRNA pool (Criterion IIa-c). This finding confirms that mimosine does not stop mRNA synthesis in general [33]. Even if bands disappeared from the polysomes (Criterion I) and also vanished from the cytoplasm (Criterion IId), certain bands reappeared within 30 min of release from mimosine (Criterion III), as shown in panel 3 of Fig. 2. Thus, on the molecular level reversibility of hypusine formation (Fig. 1, panel C) and polysomal relocalization of certain transcripts (Fig. 2, lanes p R30′ in all panels) occur simultaneously and well before resumption of proliferation can be detected. In a first approximation, we estimate that out of a total of up to 30,000 individual mRNA species in mammalian cells identifiable by the differential display technique [14,15,28], the systematic application of Criteria I–III employing mimosine will detect about 150 distinct putative *hymns*.

To establish that differentially displayed bands actually correspond to differentially translated mRNAs, the cDNA of the Class IIa double band in panel 1 was eluted, reamplified, cloned, and used for *E. coli* transformation. Following PCR

screening, two clones were sequenced and found to display an identical insert (Fig. 3A) containing the tolerably [28] mismatched arbitrary primer sequence at one end, but lacking the oligo-dT primer sequence. Referencing against the NCBI databases produced high-scoring segment pairs for both cytochrome-*c* oxidase (EC 1.9.3.1) subunit I (COX-I, [34]) ($N = 7.6$ e^{-15}) and for methionine adenosyltransferase (E.C. 2.5.1.6) (MAT, ref. [35]) ($N = 7.0$ e^{-12}) (Fig. 3B). Both COX-I and MAT, though encoded by mitochondrial and nuclear genes, respectively, show translational regulation of gene expression. In *S. cerevisiae*, COX-I translation by mitochondrial polysomes is differentially controlled via the nuclear-encoded protein NAM1p [36]; in germinating wheat embryos, MAT translation by cytoplasmic polysomes occurs from stored mRNA and proceeds unaffected by transcriptional inhibition [37]. Both COX-I and MAT also have key roles in cellular proliferation. Total COX-I transcripts are very low in quiescent cells, but increase rapidly with resumption of proliferation [38]. MAT activity directly correlates with doubling time [39]; MAT inhibition causes proliferative arrest [40]. While both proteins are translationally regulated and function in multiplication, as postulated for any putative *hymn*, identification of their mRNAs as definitive *hymn* requires further experiments to meet the three essentials described above.

The identification of a mitochondrial transcript among the putative *hymns* is unanticipated and suggests some release of mitochondrial polysomes occured during douncing. This observation points to a conceivable role of eIF-5A in directing the translation of some mitochondrial mRNAs via NAM1p, a nulear encoded protein which may be hypusine-dependent.

Others also have observed a selective effect of mimosine on critical components of proliferating cells. Under the conditions used here, mimosine was reported to abolish H1 kinase activity by selectively suppressing the formation of polyadenylated mRNAs encoding two H1 kinase components, cyclin A and *cdc2*; return of H1 kinase activity became measurable 4 h after mimosine removal, and activity normalized within 24 h [41]. A selective elimination of cyclin A, though at higher concentrations of mimosine, was noted before [20]. Using shorter exposure periods, published data show mimosine to reduce cyclin A mRNA levels [22] and to decrease $p34^{cdc2}$ [21]. Cyclin A is required for entry into S phase and onset of DNA replication [42,43], and formation of the cyclin A/$p34^{cdc2}$ complex precedes the start of S phase [21,44]. Applying the nomenclature we propose, cyclin A and *cdc2* mRNAs for these reasons might be identified among the putative class IId *hymns*.

We have recently demonstrated that cellular DOHH also is reversibly inhibited, again in concert with G1 exit, by exposure to certain mimosine fragments and either ciclopirox or transient hypoxia (H.M.H.-A., L.S.-C., R.C. Grady, M.H. Park, and J.E. Folk; unpublished observation). The latter two methods both are known to shut down G1-to-S transit [8,45–47] and replicon initiation [23,48,49]. These effects readily reverse in cultured cells, generating marked population synchrony [8,46,50–54]. The availability of ciclopirox and the employment of transient hypoxia will allow probing for Criteria I–III – defined *hymns* without reliance on mimosine. Such studies are in progress.

The identification of a sub-set of polysomal mRNAs associated with hypusine formation at the G1-S boundary adds a novel aspect to the evolutionary conservation of peptidyl hypusine formation among eukaryotes and suggests that this ability is not just a peculiarity of protein chemistry. Rather, it points to a functional integration of eIF-5A and certain ribonucleic acids that occurs in the initial stages of eukaryotic multiplication. If this functional integration is disrupted, either via its protein or its nucleic acid component, then the ability of a eukaryotic cell to control its proliferation should be compromised.

It has been reported that eIF-5A is specifically bound by the protein Rev of the human immunodeficiency virus type 1 (HIV-1) [55] and may be a crucial cellular cofactor for viral replication [56]. Without Rev, viral mRNAs are defective in their translational capacity; they appear to require eIF-5A for translation [57]. Indeed, inhibition of hypusine formation in HIV-infected cells selectively reduces viral glycoprotein biosynthesis and shifts viral mRNA away from their polysomes (L. Andrus, P.S., B.S., S.Z., R.W. Grady, A.-R.H, H.M.H.-A., unpublished observation). These findings suggest that certain HIV mRNAs may be recognized as *hymns* by the cellular translation machinery.

Experimental inhibitors of hypusine biosynthesis, at the level of either formation or hydroxylation of deoxyhypusine, i.e. DOHS or DOHH, are antiproliferative for certain normal and cancer cell lines [3,58] and also for human smooth muscle cells obtained from coronary restenotic lesions [6]. Structurally simple compounds able to interfere with hypusine synthesis are widely used already, and in each case were empirically found to modulate eukaryotic proliferation. Guazatin, which inhibits deoxyhypusine formation [59,60], has been marketed in Europe as an agricultural fungicide [61]. Mimosine has been extensively studied in Australian sheep as a defleecing agent [62]. Ciclopirox, which as predicted [8] inhibits deoxyhypusyl hydroxylation, is widely employed clinically as an excellent topical fungicide [63], its analog piroctone is a cosmetically applied anti-dandruff agent [64], and the analog rilopirox under investigation as an antimycotic [65,66]. AbeAdo, studied for its potential as an antiparasitic and antineoplastic compound, was proposed to exert its antiproliferative effect by depletion of eIF-5A [67,68]. Selective inhibition of hypusine formation by improved agents, e.g. at the level of DOHS or DOHH, may provide novel instruments for the analysis and the control of eukaryotic proliferation.

The association of hypusine formation with a sub-set of polysomal ribonucleic acids on the one hand, and with exit from the G1 phase of the cell cycle on the other, is compatible with the postulate [3] that this unique residue permits selective translation of mRNAs which encode proteins specialized to initiate and enable eukaryotic proliferation. We propose the following concept for this functional integration of eIF-5A with certain mRNAs:

Key proteins orchestrating the initiation of DNA replication and of irreversible progression through the G1-S boundary, are encoded by *hymns*, transcripts whose translation depends on appropriate hypusine formation. The consensus structure of *hymns* for interaction with the hypusine-containing domain of eIF-5A is the job-starting box (JSB): JSB defines *hymns*.

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References

[1] Park, M.H., Wolff, E.C. and Folk, J.E. (1993) Trends Biochem. Sci. 18, 475–479.
[2] Park, M.H., Wolff, E.C. and Folk, J.E. (1993) Biofactors 4, 95–104.
[3] Hanauske-Abel, H.M., Park, M.H., Hanauske, A.R., Popowicz, A.M., Lalande, M. and Folk, J.E. (1994) Biochim. Biophys. Acta 121, 115–124.
[4] Schnier, J., Schwelberger, H.G., Smit-McBride, Z., Kang, H.A. and Hershey, J.W.B. (1991) Mol. Cell. Biol. 11, 3105–3114.
[5] Abbruzzese, A., Hanauske-Abel, H.M., Park, M.H., Henke, S. and Folk, J.E. (1991) Biochim. Biophys. Acta 1077, 159–166.
[6] McCaffrey, T.A., Pomerantz, K.B., Sanborn, T.A, Spokojny, A.M., Du, B., Park, M.H., Folk, J.E., Lamberg, A., Kivirikko, K.I., Falcone, D.J., Mehta, S.B. and Hanauske-Abel, H.M. (1995) J. Clin. Invest. 95, 446–455.
[7] Hanauske-Abel, H.M. (1991) J. Hepatol. 13 (Suppl. 3), 8–16.
[8] Hoffman, B.D., Hanauske-Abel, H.M., Flint, A. and Lalande, M. (1991) Cytometry 12, 26–32.
[9] Watson, P.A., Hanauske-Abel, H.M., Flint, A. and Lalande, M. (1991) Cytometry 12, 242–2246.
[10] Lalande, M. and Hanauske-Abel, H.M. (1990) Exp. Cell Res. 188, 117–121.
[11] Lalande, M. (1990) Exp. Cell Res. 186, 332–339.
[12] Darzynkiewicz, Z., Traganos, F. and Kimmel, M. (1987) in Techniques in cell culture analysis (Gray, J.W. and Darzynkiewicz, Z., Eds.), pp. 291–336, Humana Press, Clifton.
[13] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[14] Liang, P. and Pardee, A.B. (1992) Science 257, 967–971.
[15] Liang, P., Averboukh, L. and Pardee, A.B. (1993) Nucleic Acids Res. 21, 3269–3275.
[16] Ryan, J.J., Danish, R., Gottlieb, C.A. and Clarke, M.F. (1993) Mol. Cell. Biol. 13, 711–719.
[17] Yin, Y., Tainsky, M.A., Bischoff, F.Z., Strong, L.C. and Wahl, G.M. (1992) Cell 70, 937–948.
[18] Andreassen, P.R. and Margolis, R.L. (1992) Proc. Natl. Acad. Sci. USA 89, 2272–2276.
[19] Mudzinski, S.P. (1993) Toxicol. Appl. Pharmacol. 119, 166–174.
[20] Lees, E., Faha, B., Dulic, V., Reed, S.L. and Harlow, E. (1992) Gen. Dev. 6, 1874–1885.
[21] Marracino, R.L., Firpo, E.J. and Roberts, J.M. (1992) Mol. Biol. Cell 3, 389–401.
[22] Yoshida, M., Yamamoto, M. and Nikaido, T. (1992) Cancer Res. 52, 6676–6681.
[23] Levenson, V. and Hamlin, J.L. (1993) Nucleic Acids Res. 21, 3997–4004.
[24] Perennes, C., Qin, L.X., Glab, N. and Bergounioux, C. (1993) FEBS Lett. 333, 141–145.
[25] Hamlin, J.L. (1992) BioEssays 14, 651–659.
[26] Mosca, P.J., Dijkwel, P.A. and Hamlin, J.L. (1992) Mol. Cell. Biol. 12, 4375–4383.
[27] Liang, P., Averboukh, L., Keyomarsi, K., Sager, R. and Pardee, A.B. (1992) Cancer Res. 52, 6966–6968.
[28] Bauer, D., Müller, H., Reich, J., Riedel, H., Ahrenkiel, V., Warthoe, P. and Strauss, M. (1993) Nucleic Acids Res. 21, 4272–4280.
[29] Russell, M.E., Utans, U., Wallace, A.F., Liang, P., Arceci, R.J., Karnovsky, M.J., Wyner, L.R., Yamashita, Y. and Tarn, C. (1994) J. Clin. Invest. 94, 722–730.
[30] Utans, U., Liang, P., Wyner, L.R., Karnovsky, M.J. and Russell, M.E. (1994) Proc. Natl. Acad. Sci. USA 91, 6463–6467.
[31] Kumar, R. and Haugen, J.D. (1994) Biochem. Biophys. Res. Commun. 201, 861–865.
[32] Mok, S.C., Wong, K.K., Chan, R.K., Lau, C.C., Tsao, S.W., Knapp, R.C. and Berkowitz, R.S. (1994) FASEB J. 8, 103–106.
[33] Tsai, W.C. and Ling, K.H. (1971) Toxicology 9, 241–247.
[34] Marzuki, S., Noer, A.S., Lertrit, P., Thyagarajan, D., Kapsa, R., Utthanaphol, P. and Byrne, E. (1991) Hum. Genet. 88, 139–145.
[35] Horikawa, S. and Tsukada, K. (1991) Biochem. Int. 25, 81–90.
[36] Wallis, M.G., Groudinsky, O., Slominski, P.P. and Dujardin, G. (1994) Eur. J. Biochem. 222, 27–32.
[37] Mathur, M., Saluja, D. and Sachar, R.C. (1991) Biochim. Biophys. Acta 1078, 161–170.
[38] Luciakova, K., Li, R. and Nelson, B.D. (1992) Eur. J. Biochem. 207, 253–257.
[39] Akerman, K., Karkola, K. and Kajander, O. (1991) Biochim. Biophys. Acta 1097, 140–144.
[40] Porter, C.W., Sufrin, J.R. and Keith, D.D. (1984) Biochem. Biophys. Res. Comm. 122, 350–357.
[41] Feldman, S.T. and Schönthal, A. (1994) Cancer Res. 54, 494–498.
[42] Henglein, B., Chenivesse, X., Wang, J., Eick, D. and Bréchot, C. (1994) Proc. Natl. Acad. Sci. USA 91, 5490–5494.
[43] Girard, F., Strausfeld, U., Fernandez, A. and Lamb, N.J.C. (1991) Cell 67, 1169–1179.
[44] D'Urso, G., Marraccino, R.L., Marshak, D.R. and Roberts, J.M. (1990) Science 250, 786–791.
[45] Probst, H., Schiffer, H., Gekeler, V., Kienzle-Pfeilsticker, H., Stropp, U., Stötzer, K.E. and Frenzel-Stötzer, I. (1988) Cancer Res. 48, 2053–2060.
[46] Löffler, M., Postius, S. and Schneider, F. (1978) Virchow's Arch. B Cell Path. 26, 359–368.
[47] Merz, R. and Schneider, F. (1982) Z. Naturforsch. 37c, 833–838.
[48] Probst, H., Gekeler, V. and Helftenbein, E. (1984) Exp. Cell Res. 154, 327–341.
[49] Löffler, M. (1987) Exp. Cell Res. 169, 255–261.
[50] Sinnett, D., Flint, A. and Lalande, M. (1993) Nucleic Acids Res. 21, 3227–3232.
[51] Gekeler, V. and Probst, H. (1988) Exp. Cell Res. 175, 97–108.
[52] Probst, H. and Gekeler, V. (1980) Biochem. Biophys. Res. Commun. 94, 55–60.
[53] Dreier, T., Scheidtmann, K.H. and Probst, H. (1993) FEBS Lett. 336, 445–451.
[54] Gekeler, V., Stropp, U. and Probst, H. (1986) Hoppe-Seyler's Z. Physiol. Chem. 367, 1209–1217.
[55] Ruhl, M., Himmelspach, M., Bahr, G.M., Hammerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G.K., Probst, H., Bevec, D. and Hauber, J. (1993) J. Cell Biol. 123, 1309–1320.
[56] Bevec, D., Klier, H., Holter, W., Tschachler, E., Valent, P., Lottspeich, F., Baumruker, T. and Hauber, J. (1994) Proc. Natl. Acad. Sci. USA 91, 10829–10833.
[57] Campbell, L.H., Borg, K.T., Haines, J.K., Moon, R.T., Schoenberg, D.R. and Arrigo, S.J. (1994) J. Virol. 68, 5433–5438.
[58] Park, M.H., Wolff, E.C., Lee, Y.B. and Folk, J.E. (1994) J. Biol. Chem. 269, 27827–27832.
[59] Jakus, J., Wolff, E.C., Park, M.H. and Folk, J.E. (1993) J. Biol. Chem. 268, 13151–13159.
[60] Murphey, R.J. and Gerner, E.W. (1987) J. Biol. Chem. 262, 15033–15036.
[61] Böttcher, O. (1982) in: Wirkstoffe in Pflanzenschutz- und Schädlingsbekämpfungsmitteln. Physikalisch-chemische und Toxikologische Daten (Böttcher, O., Ed.) pp. 207–208, Bintz-Verlag, Offenbach.
[62] Reis, P.J., Tunks, D.A. and Hegarty, M.P. (1975) Aust. J. Biol. Sci. 28, 495–501.
[63] Kellner, H.M., Arnold, C., Christ, O.E., Eckert, H.G., Herok, J., Hornke, I. and Rupp, W. (1981) Arzneim. -Forsch./Drug Res. 31, 1337–1353.
[64] Futterer, E. (1988) Cosmet. Toiletr. 103, 49–52.
[65] Kruse, R., Hengstenberg, W., Hanel, H. and Raether, W. (1991) Pharmacology 43, 247–255.
[66] de Angelis, H.M., Dannhorn, D.R, Hanel, H. and Kirchner, C. (1994) Arzneim. -Forsch./Drug Res. 44, 674–678.
[67] Byers, T.L., Wiest, L., Wechter, R.S. and Pegg, A.E. (1993) Biochem. J. 290, 115–121.
[68] Byers, T.L., Ganem, B. and Pegg, A.E. (1992) Biochem J. 287, 717–724.