The expansion and differentiation of hematopoietic progenitors is regulated by cytokine and growth factor signaling. To examine how signal transduction controls the gene expression program required for progenitor expansion, we screened ATLAS filters with polysome-associated mRNA derived from erythroid progenitors stimulated with erythropoietin and/or stem cell factor. The putative proto-oncogene nucleoside diphosphate kinase B (Ndph-B or nm23-M2) was identified as an erythropoietin and stem cell factor target gene. Factor-induced expression of nm23-M2 was regulated specifically at the level of polysome association by a phosphoinositide 3-kinase-dependent mechanism. Identification of the transcription initiation site revealed that nm23-M2 mRNA starts with a terminal oligopyrimidine sequence, which is known to render mRNA translation dependent on mitogenic factors. Recently, the nm23-M2 locus was identified as a common leukemia retrovirus integration site, suggesting that it plays a role in leukemia development. The expression of Nm23 from a retroviral vector in the absence of its 5′-untranslated region caused constitutive polysome association of nm23-M2. Polysome-association and protein expression of endogenous nm23-M2 declined during differentiation of erythroid progenitors, suggesting a role for Nm23-M2 in progenitor expansion. Taken together, nm23-m2 exemplifies that cytokine-dependent control of translation initiation is an important mechanism of gene expression regulation.

The expansion, survival, and differentiation of hematopoietic progenitors is largely controlled by growth factors, cytokines, and hormones. Human and murine erythroid progenitors can be expanded in vitro in the presence of erythropoietin (Epo).1

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Cytokines via Phosphoinositide 3-Kinase Signaling*

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5 The on-line version of this article (available at http://www.jbc.org) contains Fig. S1 (Differential regulation of gene expression in response to Epo and/or SCF) and Table S-I (Probes present on the ATLAS filter).
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3-kinase (PI3K) activity. Cloning of the full 5′-untranslated region (UTR) of nm23-M2 revealed that it contains both a terminal oligopyrimidine (TOP) sequence and an inverted repeat. Deletion of this 5′-UTR caused PI3K-independent, constitutive mRNA translation.

**EXPERIMENTAL PROCEDURES**

**Cells—**U11 cells were cultured in StemPro medium (Invitrogen) as described (1). For expansion, the medium was supplemented with 0.5 units/ml Epo (a kind gift of Ortho-Biotech, Tilburg, The Netherlands), 100 ng/ml SCF, and 200 ng/ml Epo and 200 mg/ml SCF. Hemoglobin accumulation was measured as described above. Samples were taken at regular intervals and processed as described previously (18). Following separation of proteins on 10% polyacrylamide gels and Western blotting, filters were incubated with antibodies against Nm23-M2 (Seikagaku, Falmouth, MA), phospho-ERK1/2 (Cell Signalling Technology, Danvers, MA), and ERK1/2 (Santa Cruz Biotechnology). Immune complexes were detected with horseradish peroxidase-conjugated goat anti-rat IgG antiserum (Santa Cruz Biotechnology) followed by an enhanced chemoluminescence reaction (PerkinElmer Life Sciences).

**Isolation and Cloning of the 5′-Untranslated Region of nm23-M2—**A nested PCR was performed on an oligo(dT)-primed cDNA library of murine erythroleukemia cells (a gift from Walbert Bakker, Department of Hematology, Erasmus MC, Rotterdam, The Netherlands). The library was ligated into the lambda ZAP express vector (Strатегane, Amster-
dam, The Netherlands). The first PCR (45 s at 94 °C, 1 min at 57 °C, and 1 min at 72 °C for 25 cycles) was performed using the M13 reverse primer (5′-ACAGAAAAACGTAGTCGTTG-3′) in the vector in combination with plN6 (5′-TGGCCCAACAGCCCCGGCCG-3′) in nm23-M2 on 50 ng of cDNA. 1 μl of PCR product was transferred to the nested PCR (45 s at 94 °C, 1 min at 56 °C, and 2 min at 72 °C for 30 cycles). The T3 primer (5′-AATTAAACTCCTACTAGGGG-3′) in the vector and plN8 (5′-TGCAAGCCACATCTGGTGTG-3′) in nm23-M2 were used for this reaction. Subsequently, the final products were cloned directly into pCR1 (Invitrogen) according to the instructions of the manufacturer. Nucleo-
tides sequencing was carried out using a binding domain sequencing kit according to instructions from the provider (PE Biosystems) Sequencing was carried out on an ABI 310 automatic sequencer (PE Biosystems) using the M13 forward primer (5′-GTAAACAGCGAGCAG-3′). All primers were obtained from Invitrogen.

**Nm23-M2 Expression Constructs—**The cDNA of nm23-M2 (NCBI accession number X68183) was cloned in the pBluescript KS vector. A triple HA tag was added of 5′ start codon of the introduced gene. Artificially introduced NcoI and BspHI restriction sites were used to isolate HA-nm23. Fragments were blunted by adding 1 unit of alkaline phosphatase (Roche Applied Science) for 15 min at 37 °C. Subsequently, the fragments were gel-purified and inserted into the PmeI site of the eukaryotic retroviral expression vector pBabe.

**Preparation of Retrovirus and Transduction of U11 Cells—**Retroviral transduction was performed as described (18). In short, ecotropic Pho-
exis cells were transfected with the use of calcium phosphate. After 24 h, cells were treated with mitomycin C (10 μg/ml) for 1 h, washed three times, and washed three more times 2 h later. U11 cells were added and co-cultured for 20–24 h in StemPro medium supplemented with Epo, SCF, and Dex as described above. Subsequently, U11 cells were re-
moved carefully from the Phoenix cells and cultured in supplemented StemPro medium. To select for stable transfectants, puromycin (2 μg/ml) was added 48 h later.

**Primer Pairs—**Gene-specific primers corresponding to nm23-M2 (X68183), rpS4 (M73436), EF-1α (BC023139), Fli-1 (X59421), and aTUB4 (M13444) (NCBI accession numbers in parentheses) were obtained from Invitrogen. The sequences of the primers used for amplification were as follows: nm23-M2, 5′-TTGGCCAACCTCTGAGC-GTAC-3′ (forward) and 5′-TTGGACCTCCCTCCAGAACACA-3′ (reverse); rpS4, 5′-TACGCCAGCTTGGTGTGCTG-3′ (forward) and 5′-TATCTC-TCCAGCTAGGCTAC-3′ (reverse); EF-1α, 5′-TATCTC-TCCAGCTAGGCTAC-3′ (forward) and 5′-TATCTC-TCCAGCTAGGCTAC-3′ (reverse); Fli-1, 5′-TGCAAGCCACATCTGGTGTG-3′ (forward) and 5′-TGCAAGCCACATCTGGTGTG-3′ (reverse); and aTUB4, 5′-TGC-GAGCTTGGTGTGCTG-3′ (forward) and 5′-TATCTC-TCCAGCTAGGCTAC-3′ (reverse).
RESULTS

Nm23-M2 Expression Is Regulated by Translational Control—Similarly to primary erythroid progenitors, the erythroid cell line I/11 proliferates in the presence of Epo, SCF, and Dex, although the cells undergo terminal differentiation into enucleated erythrocytes in medium supplemented with Epo and insulin (1, 2). We aimed to identify genes whose expression is controlled by Epo plus SCF to serve as endogenous targets in the elucidation of Epo- and SCF-specific signaling. As signal transduction may control the activation of both transcription and translation, we used polysome-bound mRNA, which was derived from I/11 erythroid cells that were factor-deprived and restimulated with Epo, SCF, or Epo plus SCF, to screen ATLAS filters containing 588 cDNA probes (2) (see also the supplementary data available in the on-line version of this article). Among other genes, we found nm23-M2 to be up-regulated by Epo, SCF, or Epo plus SCF (Fig. 1A; for complete data see the on-line supplement). The highest expression was observed when cells were stimulated with Epo plus SCF. To validate these results, we tested total RNA derived from I/11 cells that were factor-deprived and restimulated by Epo, SCF, or Epo plus SCF for nm23-M2 expression on a Northern blot. Surprisingly, we did not detect altered expression, although the Epo target gene pim1 was up-regulated by Epo, and the SCF target gene c-jun was up-regulated by SCF (Fig. 1B). Subsequently, we hybridized Northern blots containing fractions of subpolysomal and polysome-bound RNA with the nm23-M2 probe. Quantitative analysis showed that, in the absence of factor, 20% of all nm23-M2 mRNA was present in the polysomal fractions. Upon stimulation with Epo, 53% of nm23-M2 mRNA shifted into the polysomal fractions. In the presence of SCF 64% of the nm23-M2 mRNA was found in the polysomal fractions, and in the presence of Epo plus SCF the percentage was 78% (Fig. 1C). These data suggest that expression of nm23-M2 is regulated at the level of translation initiation rather than by transcriptional control.

Cytokine Regulated Polysome Association of nm23-M2 mRNA Is PI3K-dependent—The signaling routes that may be involved in growth factor-stimulated translational control are the MEK/ERK pathway, resulting in activation of p90RSK and MNK-1/2, and the PI3K/PKB pathway, activating mTOR and S6 kinase (24). To test whether these pathways are involved in mRNA Is PI3K-dependent—translational control of nm23-M2, I/11 cells were factor-depleted and subsequently SCF-stimulated in the absence or presence of the PI3K-inhibitor LY294002, the mTOR inhibitor rapamycin, or the MEK1 inhibitor PD98059. The PI3K inhibitor LY294002 completely abrogated polysome association of nm23-M2 mRNA, whereas the MEK inhibitor PD98059 did not alter the distribution of nm23-M2 transcripts as compared with untreated SCF control samples (Fig. 2A). Rapamycin only weakly reduced polysome association of nm23-M2 mRNA following SCF treatment. However, rapamycin treatment abrogated SCF-induced phosphorylation of the eIF-4E-binding protein (4E-BP), and PD98059 inhibited SCF-induced phosphorylation of ERK1/2 in parallel experiments, indicating that the quality and concentration used were effective (Fig. 2B).

Decreased nm23-M2 Protein Levels during Erythroid Differentiation Due to the Loss of Polysome Association of nm23-M2 mRNA—Previous studies reported down-regulation of Nm23-M2 protein expression in myeloid and erythroid differentiation (25, 26). Our finding that nm23-M2 mRNA associates with polysomes under erythroid “renewal” conditions is in agreement with these findings. To investigate whether nm23-M2 down-regulation was due to a change in translation, we studied nm23-M2 mRNA polysome binding in I/11 cells during terminal differentiation. I/11 cells were shifted from medium supplemented with Epo, SCF, and Dex to medium supplemented with Epo plus insulin. I/11 cells differentiated synchronously as shown previously (1, 27); cell volume is gradually reduced, and hemoglobin accumulation is accelerated between 36 and 48 h after differentiation induction (Fig. 3A). Western blotting showed that the Nm23-M2 protein is down-modulated between 36 and 48 h upon differentiation induction (Fig. 3B). Northern blots containing fractions of subpolysomal and polysomal mRNA derived from cells at different stages of differentiation showed that nm23-M2 mRNA is present in large polysomes 6 and 20 h after the induction of differentiation but that polysome association is largely lost 48 h after differentiation induction (Fig. 3C). To examine whether polysome recruitment is associated with PI3K/PKB activation, we differentiated I/11 cells for 0, 24, and 48 h, factor-depleted the cells for 4 h, and restimulated with 5 units/ml Epo and 10 units/ml insulin.
for 10 min. Both Epo and insulin induced comparable phosphorylation of PKB in expanding progenitors. Following 24 h differentiation, insulin-induced phosphorylation of PKB was increased, which may explain the increased polysome recruitment of \textit{nm23-M2} at this stage of differentiation (Fig. 3, \textbf{C} and \textbf{D}). As observed previously (18), PKB phosphorylation is downregulated 48 h following differentiation, as expression is strongly decreased (Fig. 3\textbf{D}). The loss of polysome association 48 h after differentiation induction coincides with major changes in the gene expression program of the cell (2).

\textit{Nm23-M2} mRNA Starts with a TOP Tract and Contains an Inverted Repeat—Translational regulation may occur through specific sequences and structures present in the 5′- or 3′-UTR of the mRNA. We obtained 5′-fragments of \textit{nm23-M2} cDNA from a cDNA library by PCR. Fragments of –170 bp, primed from the ATG in \textit{nm23-M2} to the 5′-cloning site of the vector, were isolated and cloned. Three independent clones were sequenced, which started at their 5′-site with a pyrimidine stretch of 8, 11, and 13 nucleotides, respectively. These clones were identical to a cDNA submitted by the RIKEN Genomic Sciences Center (AKO12447),\textsuperscript{2} which had a 12-nucleotide TOP sequence (Fig. 4). TOP tracts are structures known to be essential in the control of growth factor-dependent trans-

\footnote{\textsuperscript{2} Y. Hayashizaki, unpublished data, submission date July 10, 2000.}

\begin{figure}[h]
\centering
\includegraphics[width=\columnwidth]{fig2}
\caption{Translation of \textit{nm23-M2} is PI3K-dependent. \textbf{A}, a culture of I/11 cells was factor-deprived for 4 h and treated for 2 h with SCF (200 ng/ml) in the absence (SCF) or presence of the MEK inhibitor PD98059 (25 μM; SCF/PD), the PI3K inhibitor LY294002 (15 μM; SCF/LY), or the mTOR inhibitor rapamycin (20 ng/ml; SCF/Rapa). RNA isolated from fractions of the sucrose gradient was tested for the presence of \textit{nm23-M2}. Percentages of polysome-bound \textit{nm23-M2} mRNA indicated at the right of the panel. Lowest section, representative staining for ribosomal RNA by ethidium bromide (SCF sample). \textbf{B}, the same I/11 culture was factor-deprived for 4 h in the absence or presence of the same inhibitors at the same concentration but stimulated with SCF for 10 min. Protein lysate was analyzed on Western blot for Thr202/Tyr204-phosphorylated ERK1/2 (P-Erk), total ERK (Erk), and 4E-BP. The 4E-BP band with the highest mobility represents unphosphorylated 4E-BP, whereas the bands with a lower mobility (only present in the absence of an inhibitor or in the presence of PD98059) represent phosphorylated and hyperphosphorylated 4E-BP. LY, LY294002; Rapa, rapamycin; PD, PD98059;}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\columnwidth]{fig3}
\caption{\textit{Nm23-M2} translation is up- and down-regulated early and late during erythroid differentiation. I/11 cells were induced to differentiate by exposure to Epo and insulin. \textbf{A}, at 12-h intervals, cell size (fl; triangles) and the accumulation of hemoglobin (Hb) per cell volume (arbitrary units (a.u.); circles) were determined. \textbf{B}, cells were harvested at the times indicated, and protein samples were analyzed for \textit{Nm23-M2} expression on Western blots using \textit{α}-actin (\textit{actin}) as a loading control. \textbf{C}, polysome-bound mRNA was isolated at the time points indicated after differentiation induction. RNA isolated from 20 distinct fractions of a sucrose gradient (concentration gradient indicated below) was tested for \textit{nm23-M2} mRNA abundance (0 h, 37.5%; 6 h, 68%; 20 h, 58%; 48 h, 28%). Lowest section, representative staining for ribosomal RNA by ethidium bromide (0 h sample). \textbf{D}, I/11 cells differentiated for 0, 24, and 48 h were factor-deprived (4 h) and stimulated with 5 units/ml Epo (\textit{E}) or 10 units/ml insulin (\textit{i}) for 10 min. Cell lysates were analyzed on Western blot for phosphorylated PKB (P-PKB), total PKB (PKB), and ERK1/2 (Erk).}
\end{figure}
The 5'-UTR of nm23-M2 mRNA contains a TOP tract and an inverted repeat. The 5’-UTR of nm23-M2 mRNA was cloned by reverse transcription PCR from a cDNA library of I/11 cells. Three independent clones were sequenced, and their respective 5’-ends are indicated by vertical arrows above the sequence. The open arrow below the underlined sequence is the start of cDNA AK012447, which has been deposited to the database of the RIKEN Genomic Sciences Center. The cDNA of nm23-M2 started with a stretch of pyrimidines (italics and underlining). The 5’-UTR contains a 9-nucleotide inverted repeat creating a potential stem-loop structure as indicated with a predicted free energy of ~15.4 kcal/mol. Within the gene, the TOP and potential stem-loop sequence are separated from the translation start site by an intron of 214 nucleotides (arrow below the sequence). The protein-coding region is marked in bold, and the encoded amino acids are shown. nt, nucleotide.

Fig. 4. Mapping of the novel 5’-cDNA sequence to genomic DNA revealed that the 90 most 5’ nucleotides of the mRNA are located on a separate exon that is separated from the second exon from which the nm23-M2 open reading frame starts by an intron of 241 nucleotides. The TOP tract maps to a genomic sequence of 14 pyrimidines.

Translation of nm23-M2 mRNA Expressed from a Retroviral Vector Is Cytokine-independent—To examine whether the TOP sequence and the inverted repeat in the 5’-UTR of nm23-M2 determine the efficiency of polysome association, I/11 cells were transduced with a pBabe HA-Nm23-M2 retroviral expression construct in which the 5’-UTR is replaced by the HA tag sequence. HA-Nm23-M2-expressing clones were factor-deprived and restimulated with Epo and SCF, and RNA was separated into subpolysomal and polysomal fractions that were assayed for endogenous and exogenously expressed nm23-M2 mRNA. The polysome-associated fraction of HA-nm23-M2 mRNA was refractory to factor deprivation, in contrast to the endogenous mRNA that was consistently only present in the polysome-associated fractions when cells were restimulated with Epo/SCF (Fig. 5). Upon restimulation of the cells with Epo and SCF in the presence of LY294002 or rapamycin, polysome association of endogenous nm23-M2 mRNA was lost, but polysome association of HA-nm23-M2 was not altered. This shows that the 5’-UTR sequences are responsible for PI3K-dependent regulation of the nm23-M2 mRNA translation.

PI3K-dependent Polysome Association of RpS4, EF1B2, and nm23-M2 mRNA—We next compared the translation of nm23-m2 to other mRNAs that meet the following criteria: (i) are known to possess a TOP-tract i.e. ribosomal protein S4 (RpS4) and elongation factor 1β (EF1β); (ii) are known to be regulated at the level of translation by a very different mechanism in other cell systems, i.e. FLI-1 (29); and (iii) are known to be constitutively translated i.e. α tubulin. The cells were treated as described above, and the subpolysomal and polysomal fractions were isolated, pooled into two fractions, and transcribed into cDNA. Real time PCR (TaqMan; PE Applied Biosystems) was performed to quantitatively determine mRNA expression (Fig. 6). RpS4, EF1B2, and nm23-M2 mRNAs showed a significant shift from the subpolysomal to the polysomal RNA pool in response to Epo/SCF restimulation. The increase in polysome-associated mRNA was 10-fold for nm23-m2 and 4-5-fold for RpS4 and EF1B2. Furthermore, polysome association of nm23-M2, RpS4, and EF1B2 was almost completely abrogated by the PI3K inhibitor LY294002 and clearly reduced by rapamycin. In contrast, Epo/SCF induced only a minor increase in polysome association of FLI-1 or α-tubulin mRNA (<2-fold), and the presence of LY294002 or rapamycin did not affect polysome association of these transcripts more than what would be expected from general metabolic activation via the eIF-4E pathway. In conclusion, the TOP sequences present in the mRNAs of various translationally regulated genes (RpS4, EF1B2, and nm23-M2) renders the mRNAs sensitive to PI3K-dependent mRNA translational control. As polysome recruitment of nm23-M2 is regulated more tightly than RpS4 and EF1B2, the inverted repeat in the 5’-UTR of nm23-M2 likely contributes to factor-dependent mRNA translation.

DISCUSSION

Expression of the putative oncogene nm23-M2 in erythroid progenitors is controlled by Epo and SCF, factors that induce renewal in erythroid progenitors. Here, we demonstrate that mRNA translation is strictly controlled by pathways dependent on PI3K and mTOR, whereas nm23-M2 gene transcription is constitutively high. The nm23-M2 transcript starts with an oligopyrimidine tract and contains an inverted repeat, structures known to invoke control of translation by growth factors (28, 30). This TOP hairpin structure was shown to be essential for translational control of the nm23-M2 gene.

Regulation of Nm23-M2 mRNA Translation—Control of mRNA translation is increasingly recognized as an important additional regulatory mechanism in gene expression regulation. Internal ribosomal entry sites were found in eukaryotic mRNA and are (for instance) frequent present in mRNAs of genes involved in apoptosis to specifically assure the translation of these mRNAs under conditions that impair cap-dependent translational initiation of mRNA. Because the synthesis of ribosomes is extremely energy consuming, translation of ribosomal proteins and a number of translation factors is restricted...
HA-nm23-M2

| fraction | polysome bound |
|----------|----------------|
| NF       | 65%            |
| EPO/SCF  | 75%            |
| EPO/SCF/LY | 74%        |
| EPO/SCF/Rapa | 75%   |
| nm23-M2   |                |
| NF       | 23%            |
| EPO/SCF  | 86%            |
| EPO/SCF/LY | 28%         |
| EPO/SCF/Rapa | 54%   |

**Fig. 5.** Deletion of the 5′-UTR abrogates translational control of nm23-M2 mRNA by SCF. The 5′-UTR of nm23-M2 was substituted with a short artificial sequence followed by the sequence encoding an HA tag. HA-Nm23 expressing I/11 cells were factor-deprived (NF) and restimulated with Epo and SCF in the absence (Epo/SCF) or presence of 15 μM LY294002 (Epo/SCF/LY) or 20 ng/ml rapamycin (Epo/SCF/Rapa). RNA isolated from fractions of the sucrose gradient was tested for the presence of nm23-M2 by Northern blot analysis using a nm23-M2-specific probe. HA-nm23-M2 (top four sections) can be discriminated from endogenous nm23-M2 (middle four sections) by its slower electrophoretic mobility. Percentages of polysome-bound nm23 M2 mRNA are indicated at the right of the sections. Lowest section, representative staining for ribosomal RNA by ethidium bromide (SCF sample). LY, LY294002; Rapa, rapamycin.

PI3K-dependent Translation of Nm23-M2 mRNA

**TABLE 1.**

| Treatment | Polysome Bound |
|-----------|----------------|
| NF        | 65%            |
| EPO/SCF   | 75%            |
| EPO/SCF/LY | 74%        |
| EPO/SCF/Rapa | 75%   |
| nm23-M2   |                |
| NF        | 23%            |
| EPO/SCF   | 86%            |
| EPO/SCF/LY | 28%         |
| EPO/SCF/Rapa | 54%   |

The nm23 Gene Family and Malignant Transformation—

Enhanced expression of the nm23 family of genes has been detected in a variety of malignancies (42–46) and frequently correlated with a poor differentiation stage of certain tumors (42). Although this observation was shown for both mRNA and protein levels, the relation between mRNA and protein levels was rarely addressed. Therefore, it is not clear whether aberrant control of nm23-M2 mRNA translation is involved

interaction with RNA-binding proteins. For example, the stem-loop structure in ferritin mRNAs, the “iron responsive element,” is stabilized by a protein whose association with the mRNA is controlled by iron (30). Similarly, the potential stem-loop structure in nm23-M2 may be stabilized by a protein whose association may be dependent on its phosphorylation status. Several proteins have been shown to be able to interact with the 5′-UTR of mRNAs, for instance P56 (33, 34), the La autoantigen (35), and the cellular nucleic acid-binding protein (CNBP) (36). Ro60 is involved in the mutually exclusive binding of La or CNBP to 5′-UTRs (37). We do not yet know whether the TOP sequence and the inverted repeat in nm23-M2 mRNA both contribute to its translational control. A comparison with known TOP mRNAs that contain no other regulatory domains show that nm23-M2 mRNA translation is more strictly controlled by growth factor signaling than is the translation of ribosomal protein S4 and elongation factor-1α (EF1β). This finding suggests that both types of regulatory mechanisms may contribute to the translational control of nm23-M2.

PI3K Regulates nm23-M2 mRNA PolySome Association—

Translation of most TOP mRNAs is under the control of mTOR and PI3K-dependent pathways (38). Consistent with this fact, the translation of nm23-M2 mRNA is fully repressed upon the inhibition of PI3K. During differentiation, nm23-M2 mRNA is released from polysomes 48 h after differentiation induction concurrent with the loss of PKB activity (18). However, regulation via the inhibition of mTOR by rapamycin appears to be less stringent, similar to that of EF1β and rpS4, whose translation is also less efficiently repressed by rapamycin. Therefore, the effectiveness of LY294002 compared with that of rapamycin may be specific for erythroid progenitors and/or the conditions used rather than being a property of nm23-M2 mRNA. PI3K activates PKB and mTOR, which results in phosphorylation of eIF-4E-binding proteins (4E-BP) and the release of eIF-4E. Increased levels of available eIF-4E are thought to facilitate scanning of the translation initiation complex past structural obstacles like TOP sequences and hairpin structures. In addition, the PI3K pathway may result in the phosphorylation and release of RNA-binding proteins that stabilize the mRNA secondary structure, although such mechanisms have not yet been identified. In erythroid progenitors, the cooperation of Epo with SCF induces renewal and delays differentiation (39). The inhibition of PI3K abrogates renewal and induces differentiation (1), whereas inhibition of the MEK/ERK pathway does not affect the balance between expansion and differentiation. This suggests that PI3K targets like nm23-M2 may be involved in renewal induction. Accordingly, the enhanced activity of PI3K and its downstream targets is also crucial in tumors induced by a mutated PI3K (v-p3k) functioning as an oncogene in avian sarcoma virus 16 (ASV16)-induced tumors (40) and in Friend spleen focus-forming virus-induced erythroleukemia (41). However, limited attempts to show the effects of Nm23 overexpression in murine I/11 erythroblasts failed to detect clear effects of Nm23 on renewal, survival, or factor dependence. Thus, Nm23 may function in myeloid or multipotent cells rather than in erythroid cells and/or may require cooperation with other, to date unknown, oncogenic events to cause leukemia.

The nm23 Gene Family and Malignant Transformation—

Enhanced expression of the nm23 family of genes has been detected in a variety of malignancies (42–46) and frequently correlated with a poor differentiation stage of certain tumors (42). Although this observation was shown for both mRNA and protein levels, the relation between mRNA and protein levels was rarely addressed. Therefore, it is not clear whether aberrant control of nm23-M2 mRNA translation is involved
in altered nm23 protein expression in malignancy. Most likely, transcriptional as well as translational control mechanisms regulate nm23-M2 expression. In retrovirally induced mouse leukemias, integrations occur both upstream and downstream of the nm23-m2 ORF (14). Many integrations, however, occurred downstream of the translation initiation site and upstream of the ATG start codon. In these cases, the retroviral long terminal repeat could act as a promoter element to give rise to a fusion transcript in which the translational control elements of nm23-M2 (TOP sequence and repeat) are replaced with viral sequences that ensure rapid scanning of the translation initiation complex. As a result, viral integration may abolish regulation of nm23-M2 expression by growth factors/cytokines and, thus, promote leukemogenesis. Unfortunately, it appears to be impossible to isolate polysome-associated mRNA from stored primary mouse leukemia samples. Therefore, the effect of virus integration on the translational regulation of nm23-M2 in the leukemias containing long terminal repeat-nm23-M2 fusion transcripts will have to await future analysis.

Oncogenesis and Translational Control—Control of translation initiation via mRNA-specific mechanisms is increasingly recognized as a potentially important control level in lineage determination, cell survival, proliferation, differentiation, and disease (47, 48). The oncogenic role of Ras and PKB in glioblastoma was shown to involve differential recruitment of existing translation initiation complexes. As a result, rapid changes in protein levels. For example, the expression of many proto-oncogenes is associated with multiple, virally induced leukemias in mouse, and the human counterpart is translocated in Ewing tumors (55). Several mechanisms for retrovirally induced malignant transformation have, to date, been identified, ranging from the activation of transcription to mRNA stabilization or gene inactivation. Data presented in this paper suggest that an important mechanism may so far have been underestimated, namely the replacement of untranslated sequences important for translational control in mRNAs encoding potential oncogenes such as nm23, leading to loss of such translational control by growth factors/cytokines.

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