

## Growth Factors in the Nucleolus?

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**I**N eukaryotic cells containing tandem repeated ribosomal RNA genes, there appears a specialized region of chromatin, carrying out gene transcription, rRNA processing, and nascent ribosomal subunit assembly—the nucleolus. One of the first intracellular structures to be described (Montgomery, 1898; Franke, 1988), the nucleolus was established in the 1960s as the center of ribosome synthesis (Schultz, 1966), culminating in the first visualization of genes in action (Miller and Beatty, 1969). Recently it has become evident, however, that the nucleolus is also the site of several nonribosomal RNA processing and assembly functions. These include maturation of signal recognition particle RNA (Jacobson and Pederson, 1998), transfer RNA, U6 small nuclear RNA, telomerase RNA, and some mRNAs (Pederson, 1998). In the envisioned proto-eukaryotic ancestor, various RNA processing and ribonucleoprotein assembly events may have all initially been centered around a minimal genome to chemically facilitate the production of essential gene readout machines. The nucleolus of today's eukaryotes may be a descendent of this concentrated region of genome readout. Beyond these recently discovered, diverse nonribosomal RNA processing and ribonucleoprotein assembly events in the nucleolus, there is another set of provocative findings: the presence in nucleoli of mitogenic growth factors as well as cell cycle and growth factor-related proteins.

### *Growth Factors and Growth Regulatory Proteins in the Nucleolus*

Although there are numerous studies (of varying quality and cogency) indicating the presence in nuclei of polypeptide growth factors and, in a few instances, growth factor receptors (Burwen and Jones, 1987; Jans, 1994; Stachowiak et al., 1997), the more salient point for this commentary is that during the past few years a number of mitogenic growth factors and other growth regulatory proteins have been observed to be localized in the nucleolus. These include basic fibroblast growth factor (Bouche et al., 1987; Baldin et al., 1990; Moroianu and Riordan, 1994), acidic fibroblast growth factor (Moroianu and Riordan, 1994), an-

giogenin (Moroianu and Riordan, 1994), parathyroid hormone-related peptide (pTHrP)<sup>1</sup> (Henderson et al., 1995; Nguyen and Karaplis, 1998), the Werner syndrome (WRN) gene product (Marciniak et al., 1998), the B-type cyclin p63<sup>cdc13</sup> (Gallagher et al., 1993), and the oncogene *c-myc*-associated protein p160 (Tavner et al., 1998). Although this list seems brief at first glance, it is actually rather impressive when one considers that most surveyors of the nucleolar protein landscape have had no particular motivation to look for growth factors altogether.

Several of these aforementioned studies involved incubating cells with exogenous growth factors, ostensibly approximating the *in vivo* paracrine/autocrine situation. However, the cited study of pTHrP involved its transient expression and nucleolar localization (Henderson et al., 1995) and, in addition, these investigators observed that endogenous pTHrP was present in nucleoli of cultured neonatal osteoblasts. There are precedents in which alternative mRNA splicing or a switch in initiation codon leads to the production of a growth factor lacking its normal secretory signal peptide and, in some instances, subsequent nuclear localization (Maher et al., 1989; Acland et al., 1990; Quarto et al., 1991; Vagner et al., 1996). The case of pTHrP illustrates that this “intracrine” pathway can lead not only to nuclear translocation but also localization in the nucleolus.

### *Tyrosine Phosphorylation, a Hallmark of Growth Factor Signal Transduction, Also Occurs in the Nucleolus As Does Other Signal Transduction-related Protein Phosphorylation*

Among numerous reports of protein tyrosine phosphorylation/dephosphorylation reactions in the nucleus altogether (David et al., 1993; McLaughlin and Dixon, 1993; Rohan et al., 1993), there are two reports of tyrosine phosphorylation of specifically nucleolar proteins: the yeast immunophilin Fpr3 (Wilson et al., 1997), and the protozoan *Trypanosoma brucei* proteins Nopp44/46 (Das et al., 1996). The tyrosine phosphorylation of Fpr3 is mediated by casein kinase II (Wilson et al., 1997), an enzyme that usually phosphorylates only serine or threonine in higher eukaryotes but which also has specificity for tyrosine in

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1. Abbreviation used in this paper: pTHrP, parathyroid hormone-related peptide.

both *Saccharomyces cerevisiae* and *Saccharomyces pombe*. The immunophilin family of which the yeast nucleolar Fpr3 protein is a member is defined by the immunosuppressive drug FK506 and its numerous intracellular targets (Sigel and Dumont, 1992), and it is noteworthy in the present context that one of these targets, the protein FKB25, is known to associate both with casein kinase II and the nucleolar protein nucleolin (Jin and Burakoff, 1993).

Of course, two mere instances of tyrosine phosphorylation of nucleolar proteins do not, by themselves, provide anything more than a zephyr of a connection to the nucleolus as a center of mitogenic growth factor action, although the Fpr3-FKB25–nucleolin connection is certainly provocative. In addition to tyrosine phosphorylation of nucleolar proteins, it has been observed that basic fibroblast growth factor binds to the  $\beta$  subunit of casein kinase II, stimulating the serine/threonine phosphorylation of nucleolin (Bonnet et al., 1996). Moreover, the nuclear envelope phosphoinositide-based protein phosphorylation system (Martelli et al., 1992; Divecha et al., 1993; Raben and Jaken, 1994) displays multiple isoforms of protein kinase C (PKC), some of which are nucleolar (Beckmann et al., 1994). Indeed, one of the targets of nerve growth factor-triggered PKC phosphorylation is, once again, nucleolin (Zhou et al., 1997), and in a recent investigation evidence was reported that neomycin blocks nuclear translocation of angiogenin by virtue of this antibiotic's inhibitory action on phospholipase C (Hu, 1998).

An intriguing observation in relation to signal transduction-related phosphorylation of nucleolar proteins is the finding that the aforementioned yeast nucleolar FK506 binding protein has homology with a protozoan (*Naegleria gruberi*) nucleolar protein, BN46/51 (Goekeler et al., 1997), which is also found in the *Naegleria* basal body (Trimbur and Walsh, 1992). The basal body is a specialized centriole that nucleates the assembly of flagellar and ciliary microtubules into the 9 + 2 motif of the axoneme. (*Naegleria* shifts from ameboid locomotion to flagellar-propelled swimming during its life cycle.) The basal body is an autonomously replicating organelle, in which the presence of a nucleic acid component has been long sought unsuccessfully. That the nucleolus and basal body of *Naegleria* share a common protein is itself interesting with regard to the evolution of these two organelles, and the fact that this protein shares homology with the yeast nucleolar FK506-binding protein serves to raise the possibility of a connection to signal transduction pathways.

### Perspective

There is rarely anything to lose from thinking in the context of evolution. The typical growth factors we know today arose, or were refined, with the advent of metazoan life, evolving (probably from ancient chemoattractants) as ligands in step with their coemerging cell surface receptors. However, some of today's secreted growth factors might be evolutionarily descended from proteins that were once capable of triggering cell division from within, and indeed, as mentioned above, such intracrine pathways appear to operate in extant eukaryotes. In the single-cell forebears of metazoa, it is likely that intracellular signal

proteins would have already been hewn to target on genomic sites at which growth-promoting genes resided. Such intracellular signaling in the predecessors of the metazoa may have embraced protein tyrosine phosphorylation and may have also shared elements with other replicating domains of the cell in addition to the nuclear genome, the basal body for example. Today, more than two billion years later (Han and Runnegar, 1992), we properly see growth factors as primarily operatives on the cell surface, binding receptors in the intercellular signaling life-style that defines metazoan life, notwithstanding concurrently operating intracrine pathways as well.

How do nucleolus-localized growth factors actually work? Of course, all roads that lead to the nucleolus would logically suggest ribosome synthesis as the obvious target of regulation. For example, fibroblast growth factor-mediated phosphorylation of nucleolin has been implicated in turning on ribosome production (Bouche et al., 1987; Bonnet et al., 1996). It is certainly true that the rate of rRNA gene transcription, pre-rRNA processing, and nascent ribosome subunit assembly—the canonical roles of the nucleolus—could be downstream elements in positively controlling cell growth, although the typically long half-life of cytoplasmic ribosomes (Loeb et al., 1965) might often emerge as a limiting parameter in negative growth control loops. But the recent reports that other, nonribosomal RNA maturation events also occur in the nucleolus (Jacobson and Pederson, 1998; Pederson, 1998) now suggest other targets of growth control therein. The presence of growth factors and cell cycle-related proteins in the nucleolus currently constitutes something of an intellectual way station, neither an established piece of orthodoxy on the one hand nor necessarily an opaque box on the other. We need to know how these nucleolus-localized growth factors operate in gene readout, as the era of the plurifunctional nucleolus now comes into view.

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### References

- Acland, P., M. Dixon, G. Peters, and C. Dickson. 1990. Subcellular fate of the Int-2 oncoprotein is determined by choice of initiation codon. *Nature*. 343: 662–665.
- Baldin, V., A.-M. Roman, I. Bosc-Breine, F. Almaric, and G. Bouche. 1990. Translocation of bFGF to the nucleus is G<sub>1</sub> phase cell cycle specific in bovine aortic endothelial cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1511–1517.
- Beckmann, R., C. Lindschau, H. Haller, F. Hucho, and K. Buchner. 1994. Differential nuclear localization of protein kinase C isoforms in neuroblastoma × glioma hybrid cells. *Eur. J. Biochem.* 222:335–343.
- Bonnet, H., O. Filhol, I. Truchet, P. Brethenou, C. Cochet, F. Almaric, and G. Bouche. 1996. Fibroblast growth factor-2 binds to the regulatory  $\beta$  subunit of CK2 and directly stimulates CK2 activity toward nucleolin. *J. Biol. Chem.* 271:24781–24787.
- Bouche, G., N. Gas, H. Prats, V. Baldin, J.-P. Tauber, J. Teissie, and F. Almaric. 1987. Basic fibroblast growth factor enters the nucleolus and stimulates the transcription of ribosomal genes in ABAE cells undergoing G<sub>0</sub>→G<sub>1</sub> transition. *Proc. Natl. Acad. Sci. USA.* 84:6770–6774.

- Burwen, S.J., and A.L. Jones. 1997. The association of polypeptide hormones and growth factors with the nuclei of target cells. *Trends Biochem. Sci.* 12: 159–162.
- Das, A., G.C. Peterson, S.B. Kanner, U. Frevert, and M. Parsons. 1996. A major tyrosine-phosphorylated protein of *Trypanosoma brucei* is a nucleolar RNA-binding protein. *J. Biol. Chem.* 271:15675–15681.
- David, M., P.M. Grimley, D.S. Finbloom, and A.C. Lerner. 1993. A nuclear tyrosine phosphatase downregulates interferon-induced gene expression. *Mol. Cell. Biol.* 13:7515–7521.
- Divecha, N., H. Banfic, and R.F. Irvine. 1993. Inositides and the nucleus and inositides in the nucleus. *Cell.* 74:405–407.
- Franke, W.W. 1988. Matthias Jacob Schleiden and the definition of the cell nucleus. *Eur. J. Cell Biol.* 47:145–156.
- Gallagher, I.M., C.E. Alfa, and J.S. Hyams. 1993. p63<sup>cdc13</sup>, a B-type cyclin, is associated with both the nucleolar and chromatin domains of the fission yeast nucleus. *Mol. Biol. Cell.* 4:1087–1096.
- Goekeler, J., J.L. Brodsky, and C. Walsh. 1997. Cloning, sequencing and expression of the MTOC associated nucleolar protein BN46/51 reveals similarity to FK506 binding proteins. *Mol. Biol. Cell.* 8:217a.
- Han, T.-M., and B. Runnegar. 1992. Megascopic eukaryotic algae from the 2.1-billion-year-old Negaunee iron-formation, Michigan. *Science.* 257:232–235.
- Henderson, J.E., N. Amizuka, H. Warshawsky, D. Biasotto, B.M.K. Lanske, D. Goltzman, and A.C. Karaplis. 1995. Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. *Mol. Cell. Biol.* 15:4064–4075.
- Hu, G.-F. 1998. Neomycin inhibits angiogenin-induced angiogenesis. *Proc. Natl. Acad. Sci. USA.* 95:9791–9795.
- Jacobson, M.R., and T. Pederson. 1998. Localization of signal recognition particle RNA in the nucleolus of mammalian cells. *Proc. Natl. Acad. Sci. USA.* 95:7981–7986.
- Jans, D.A. 1994. Nuclear signaling pathways for polypeptide ligands and their membrane receptors. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 8:841–847.
- Jin, Y.J., and S.J. Burakoff. 1993. The 25-kDa FK506-binding protein is localized in the nucleus and associates with casein kinase II and nucleolin. *Proc. Natl. Acad. Sci. USA.* 90:7769–7773.
- Loeb, J.R., R.R. Howell, and G.M. Tomkins. 1965. Turnover of ribosomal RNA in rat liver. *Science.* 14:1093–1095.
- Maher, D.W., B.A. Lee, and D.J. Donoghue. 1989. The alternatively spliced exon of the platelet-derived growth factor A chain encodes a nuclear targeting signal. *Mol. Cell. Biol.* 9:2251–2253.
- Marciniak, R.A., D.B. Lombard, F.B. Johnson, and L. Guarente. 1998. Nucleolar localization of the Werner syndrome protein in human cells. *Proc. Natl. Acad. Sci. USA.* 95:6887–6892.
- Martelli, A.M., R.S. Gilmour, V. Bertagnolo, L.M. Neri, L. Manzoli, and L. Cocco. 1992. Nuclear localization and signalling activity of phosphoinositidase C<sub>β</sub> in Swiss 3T3 cells. *Nature.* 358:242–245.
- McLaughlin, S., and J.E. Dixon. 1993. Alternative splicing gives rise to a nuclear protein tyrosine phosphatase in *Drosophila*. *J. Biol. Chem.* 268:6839–6842.
- Miller, O.L., Jr., and B.R. Beatty. 1969. Visualization of nucleolar genes. *Science.* 164:955–957.
- Montgomery, T.H. 1898. Comparative cytological studies, with special regard to the morphology of the nucleolus. *J. Morphology.* 15:265–582.
- Moroianu, J., and J.F. Riordan. 1994. Nuclear translocation of angiogenin in proliferating endothelial cells is essential to its angiogenic activity. *Proc. Natl. Acad. Sci. USA.* 91:1677–1681.
- Nguyen, M.T.A., and A.C. Karaplis. 1998. The nucleus: a target site for parathyroid hormone-related peptide (pTHrP) action. *J. Cell. Biochem.* 70:193–199.
- Pederson, T. 1998. The plurifunctional nucleolus. *Nucleic Acids Res.* 26:3871–3876.
- Quarto, N., F.P. Finger, and D.B. Rifkin. 1991. The NH<sub>2</sub>-terminal extension of high molecular weight bFGF is a nuclear targeting signal. *J. Cell. Physiol.* 147:311–318.
- Raben, D.M., M.B. Jarpe, and K.L. Leach. 1994. Nuclear lipid metabolism in NEST: nuclear envelope signal transduction. *J. Membr. Biol.* 142:1–7.
- Rohan, P.J., P. Davis, A. Moskaluk, M. Kearns, H. Krutzsch, U. Siebenlist, and K. Kelly. 1993. PAC-1: a mitogen-induced nuclear protein tyrosine phosphatase. *Science* 259:1763–1766.
- Schultz, J. 1966. Perspectives for the conference on the nucleolus. *Natl. Cancer Inst. Monogr.* 23:1–9.
- Sigel, N.H., and F.J. Dumont. 1992. Cyclosporin A, FK506 and rapamycin: pharmacologic probes of lymphocyte signal transduction. *Annu. Rev. Immunol.* 10:519–560.
- Stachowiak, E.K., P.A. Maher, J. Tucholski, E. Mordecai, A. Joy, J. Moffett, S. Coons, and M.K. Stachowiak. 1997. Nuclear accumulation of fibroblast growth factor receptors in human glial cells—association with cell proliferation. *Oncogene.* 14:2201–2211.
- Tavner, F.J., R. Simpson, S. Tashiro, D. Favier, N.A. Jenkins, D. Gilbert, N.G. Copeland, E.M. Macmillan, J. Lutwyche, R.A. Keough, S. Ishii, and T.J. Gonda. 1998. Molecular cloning reveals that the p160 myb-binding protein is a novel, predominantly nucleolar protein which may play a role in transactivation by myb. *Mol. Cell. Biol.* 18:989–1002.
- Trimbur, G.M., and C.J. Walsh. 1992. BN46/51, a new nucleolar protein, binds to the basal body region in *Naegleria gruberi* flagellates. *J. Cell Sci.* 103:167–181.
- Vagner, S., C. Touriol, B. Galy, S. Audigier, M.-C. Gensac, F. Amalric, F. Bayard, H. Prats, and A.-C. Prats. 1996. Translation of CUG- but not AUG-initiated forms of human fibroblast growth factor 2 is activated in transformed and stressed cells. *J. Cell Biol.* 135:1391–1402.
- Wilson, L.K., N. Dhillon, J. Thorner, and G.S. Martin. 1997. Casein kinase II catalyzes tyrosine phosphorylation of the yeast nucleolar immunophilin Fpr3. *J. Biol. Chem.* 272:12961–12967.
- Zhou, G., M.L. Seibenhener, and M.W. Wooten. 1997. Nucleolin is a protein kinase C-ζ substrate. Connection between cell surface signaling and nucleus in PC12 cells. *J. Biol. Chem.* 272:31130–31137.