Requirement of Nuclear Prolactin for Interleukin-2–Stimulated Proliferation of T Lymphocytes

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Prolactin (PRL) is necessary for the proliferation of cloned T lymphocytes in response to interleukin-2 (IL-2). Translocation of PRL into the nucleus occurs during IL-2–stimulated mitogenesis. Therefore, the function of intranuclear PRL in T cell proliferation was tested. Eukaryotic expression vectors were prepared to express wild-type PRL [PRL(WT)], PRL that lacks the signal sequence for translocation into the endoplasmic reticulum [PRL(ER−)], and chimeric PRL in which the signal peptide was replaced with the sequence that directs the nuclear translocation of the SV40 large T antigen [PRL(NT+)]. Expression of these constructs in a T cell line (Nb2) responsive to PRL and IL-2 resulted in localization of PRL in the extracellular milieu, cytoplasm, or nucleus, respectively. Stimulation with IL-2 alone resulted in a five- to tenfold increase in the incorporation of [^{3}H]thymidine by cells expressing PRL(NT+) or PRL(WT) as compared to PRL(ER−) or the parental Nb2 cells. Only the PRL(NT+) clone proliferated continuously with IL-2 stimulation in the presence of antiserum to PRL. These results demonstrate that nuclear PRL is necessary for IL-2–stimulated proliferation and suggest that a peptide hormone can function in the nucleus without binding to its cell surface receptor.

ONE OF THE PHYSIOLOGIC EFFECTS of the neuroendocrine hormone PRL is regulation of the immune system (*1*). Ablation of PRL secretion in vivo diminishes immune response (*2*), and proliferation of lymphocytes mediated by mitogens and lymphokines is inhibited by antiserum to PRL in vitro (*3, 4*). These data indicate that PRL may act as a lymphocyte progression factor. PRL induces the transcription of cell cycle–associated gene products in Nb2 cells, a PRL- and IL-2–responsive T lymphocyte line from rat (*5*). Although the PRL receptor has been cloned (*6*), it is not known how signal transduction is initiated by receptor binding of PRL (*7*). As IL-2–stimulated T cells pass through the G_1 phase of the cell cycle, PRL, but not the PRL receptor, is translocated into the lymphocyte nucleus (*8, 9*). One interpretation of these data is that nuclear PRL functions in stimulating progression through the cell cycle.

We expected that naturally occurring signal or translocation sequences could be used to specifically target bioactive PRL into the nucleus, cytoplasm, or extracellular milieu of Nb2 lymphocytes. Many secreted hormones, including native PRL, contain an NH_2-terminal sequence of 15 to 30 amino acids that promotes their secretion into the endoplasmic reticulum and ultimately into the extracellular space. If this sequence is removed, proteins are retained in the cytoplasm (*10*). Attachment of a small basic sequence from SV40 large T antigen to a protein can direct translocation of that protein to the nucleus (*11*). Therefore, modified genes encoding forms of PRL with altered targeting signals were synthesized in the polymerase chain reaction with the appropriate oligonucleotide primers from a full-length cDNA clone of rat PRL [PRL(WT)] (*12*). From these syntheses, two mutant PRL genes were subcloned; the PRL(ER−) clone lacked the signal sequence, and in the PRL(NT+) clone, the signal sequence was replaced with the nuclear translocation sequence from the SV40 large T antigen (Fig. 1).

Localization of native and modified forms of PRL was examined by transfecting Cos-1 cells with the PRL(WT), PRL(ER−), and PRL(NT+) constructs. Intracellular PRL was detected by indirect immunofluorescence with antiserum to PRL (anti-PRL) (Fig. 2A); extracellular PRL was detected by bioassay (*13*). Cos-1 cells transfected with PRL(WT) showed only faint immunofluorescence in the perinuclear and Golgi region and secreted biologically active PRL (approximately 100 ng/ml from 3×10^5 cells cultured for 48 hours). No PRL was detected in the culture medium of Cos-1 cells transfected with PRL(ER−) or PRL(NT+); however, accumulation of immunoreactive PRL was seen within the cytoplasm and nucleus, respectively.

The PRL constructs were subsequently transfected into Nb2 T lymphocytes. Twelve clones for each construct were obtained and showed similar amounts of PRL by immunoblot analysis. For each PRL construct, four stable transfectants were selected randomly and analyzed; representative transfectants are described below. Immunoblot analysis of each of the Nb2 clones revealed the presence of a single immunoreactive species that comigrated with purified PRL, which was not observed in the parental Nb2 line (Fig. 2B). Bioassay detected secretion of PRL only from the PRL(WT) clone, while biologically active PRL was found in the cell lysates of each of the transfected clones (Fig. 2C). Immunofluoresence assay revealed appropriate location of the PRL constructs in the Nb2 subclones (Fig. 2D).

The effect of the subcellular localization of PRL on the growth of Nb2 cells in PRL-free and IL-2–free medium was examined (Fig. 3). In the absence of exogenous PRL or IL-2, the parental Nb2 cells failed to incorporate [^{3}H]thymidine. Of the Nb2 clones, only PRL(WT) incorporated [^{3}H]thymidine in medium lacking both PRL and IL-2 (*14*). The PRL(WT) clone could be maintained in PRL- and IL-2–free medium for more than 2 months, demonstrating that the autocrine interaction of secreted PRL with its cell surface receptor is necessary and sufficient to maintain cellular proliferation in the absence of other stimuli. Conversely, the presence of biologically active PRL in the cytoplasm or nucleus was not sufficient to induce proliferation.

PRL enhances the proliferative response of Nb2 cells to stimulation by IL-2 (*13*). Because PRL is present in the nuclei of lymphocytes proliferating in vitro (*4, 9*), it is possible that nuclear PRL enhances the proliferative response of Nb2 cells to IL-2. We therefore examined the growth of the transfected Nb2 clones and the parental Nb2 cells in PRL-free medium that contained IL-2 (Fig. 4A). Cells from the PRL(ER−) clone and the parental NB2 line cultured in the presence of IL-2 incorporated two to ten times more [^{3}H]thymidine than did such cells cultured in unsupplemented medium (*13*). However, in the presence of IL-2, both the PRL(WT) and PRL(NT+) clones incorporated five to ten times more [^{3}H]thymidine than either the parental Nb2 or PRL(ER−) clones. These observations

	-29	-20	-10	1	5
PRL (WT)	MNSQVSARKAGTLLLLMMSNLLFCQNVQTLPVCS...				
PRL (ER-)	MLPVCS...				
PRL (NT+)	MPKKKRKVLPVCS...				

Fig. 1. Structure of the NH_2-terminal domain of PRL and the modified forms of PRL. Shown are the amino acid sequence of residues −29 to 5 of the secreted precursor of PRL, PRL(WT); the sequence of the truncated mutant, PRL(ER−), and the sequence of the chimera, PRL(NT+), that in place of the signal sequence contained the nuclear localization sequence of the SV40 large T antigen (*12, 19*).

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indicate that both extracellular and intranuclear PRL can synergize with IL-2 in augmenting the proliferative response of Nb2 cells. To ensure that PRL in the PRL(NT+) clone was functioning within the nucleus, and not leaking from the cell and stimulating the surface receptor, cells were cultured in the presence of both IL-2 and anti-PRL (Fig. 4B). Anti-PRL inhibited [^{3}H]thymidine incorporation in the PRL(ER−) clone and the parental Nb2 line after 1 day of culture, and in the PRL(WT) clone after 3 days. The PRL(WT) clone is continuously producing and utilizing PRL and the PRL bound to surface receptors or internalized must be depleted before proliferation can be inhibited. Because anti-PRL only removes extracellular PRL, it probably takes at least 1 day for anti-PRL to deplete the intracellular PRL that is mediating proliferation. The proliferation of the PRL(NT+) cells was not affected by the addition of anti-PRL at either time point. Together, the observations that the PRL(NT+) clone does not secrete PRL, does not incorporate [^{3}H]thymidine in the absence of exogenous PRL or IL-2, and does proliferate in response to IL-2 in the presence of anti-PRL indicate that nuclear PRL, independent of its receptor on the cell surface, is necessary for the proliferative response of Nb2 cells to IL-2. Like cloned T lymphocytes, Nb2 cells require PRL for IL-2–driven proliferation (*4, 9*). The Nb2 cells apparently require both a cell surface signal that can be provided by PRL or IL-2 to initiate cell cycle progression and some function provided by nuclear PRL that is necessary, but not sufficient, for

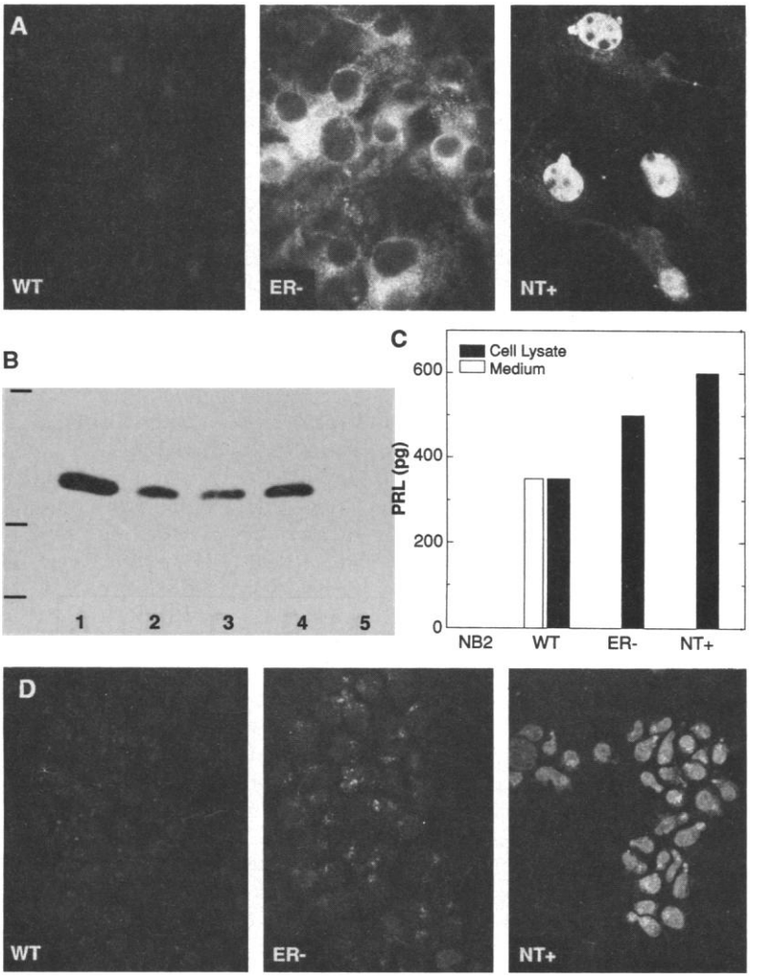

Fig. 2. Intracellular localization, characterization, and bioassay of native PRL [PRL(WT)] and its mutated forms [PRL(ER−) and PRL(NT+)]. (**A**) Transient expression in Cos-1 cells. Cos-1 cells (3×10^5) were transfected with the PRL constructs (10 μg) according to a modified DEAE-dextran-chloroquine protocol (*20*) and analyzed after 48 hours of culture by indirect immunofluorescence with anti-PRL (×500). (**B**) Detection of purified PRL (2 ng, lane 1) and PRL in cell extracts (1×10^6 washed cells were cultured for 24 hours in PRL-free medium lysed by freezing and thawing, and cleared by centrifugation) from the transfected PRL(WT), PRL(ER−), and PRL(NT+) clones and parental Nb2 cell line (lanes 2 to 5, respectively) by protein immunoblotting (*7, 14*). Densitometric quantitation in arbitrary volumetric units revealed the following values: 3.84, 1.32, 1.04, 1.95, 0.04; lanes 1–5, respectively. Molecular size standards indicated at left are 29, 18, and 14 kD, top to bottom. One of three representative blots is presented. (**C**) Bioassay of media and cell extracts from the transfected PRL(WT), PRL(ER−), and PRL(NT+) clones and parental Nb2 cells. The conditioned media were obtained by inoculating 5×10^5 cells in 1 ml of PRL-free medium for 24 hours. Bioassay of cell extracts was performed on cell extracts (see above) from 1×10^6 cells (*13, 14*). The values shown are means ± SEM. One of two representative experiments is presented. (**D**) Expression of PRL constructs in Nb2 rat T cell lymphoma cells. Nb2 cells were transfected with the PRL constructs by lipofection (*21*), subcloned by limiting dilution in the presence of G418, and analyzed by anti-PRL indirect immunofluoresence microscopy (×500). Because the Nb2 cells have less cytoplasm than Cos-1 cells, the anti-PRL immunofluorescence within the cytoplasm of PRL(ER−) Nb2 clone is reduced compared to that in Cos-1 cells transfected with PRL(ER−).

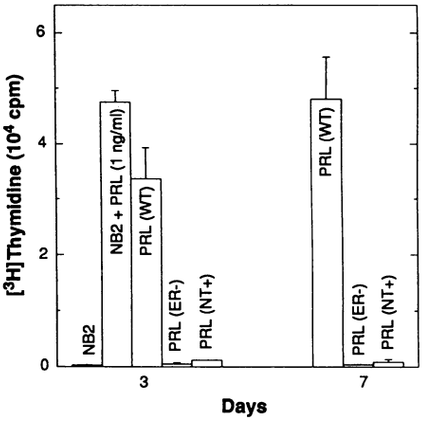

Fig. 3. Growth of the PRL(WT), PRL(ER−), and PRL(NT+) clones and Nb2 cells in medium lacking IL-2 and PRL. Cell proliferation (3×10^5 cells) was assessed by a 4-hour incubation in the presence of [^{3}H]thymidine (2 μCi/ml) on the day indicated. The values expressed are means (counts per minute) ± SEM of [^{3}H]thymidine incorporated from replicates. One of two representative experiments is presented.

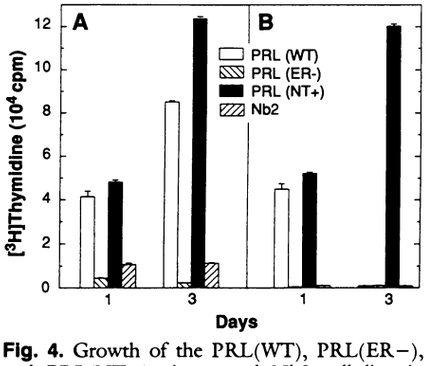

Fig. 4. Growth of the PRL(WT), PRL(ER−), and PRL(NT+) clones and Nb2 cell line in PRL-free medium containing recombinant human IL-2 (100 units/ml), in the absence (**A**) and presence (**B**) of a 1:500 dilution of anti-PRL (*4*). Cellular proliferation was assessed as in Fig. 3. [^{3}H]thymidine incorporation of the parental Nb2 line in the absence of IL-2 was 558 ± 122 cpm and 1755 ± 633 after 1 and 3 days, respectively. One of four representative experiments is presented.

cell cycle progression.

Peptide hormones other than PRL may also function within the nucleus. The mitogenic activity of fibroblast growth factor depends on its nuclear translocation sequence (*15*). Other peptide ligands that translocate into the nucleus such as IL-1 (*16*), platelet-derived growth factor (*17*), and insulin (*18*) may function in a similar manner. Further study of the internalization and intracellular interactions of PRL may define the mechanisms through which this peptide hormone influences cell cycle progression and immune responsiveness.

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22. We thank A. Sillman and N. Thornton for technical assistance, R. Maurer for the rat PRL cDNA, G. D Johnson for the RLDN(10B) vector, and the Cetus Corporation (Emeryville, CA) for the IL-2. Supported in part by grant GM-36962 (M.B.P.) from the NIH and a National Research Service Award GM-13901 (C.V.C.).

22 January 1991; accepted 24 April 1991

Deregulation of a Homeobox Gene, HOX11, by the t(10;14) in T Cell Leukemia

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Molecular cloning of the t(10;14)(q24;q11) recurrent breakpoint of T cell acute lymphoblastic leukemia has demonstrated a transcript for the candidate gene TCL3. Characterization of this gene from chromosome segment 10q24 revealed it to be a new homeobox, HOX11. The HOX11 homeodomain is most similar to that of the murine gene *Hlx* and possesses a markedly glycine-rich variable region and an acidic carboxyl terminus. HOX11, while expressed in liver, was not detected in normal thymus or T cells. This lineage-restricted homeobox gene is deregulated upon translocation into the T cell receptor locus where it may act as an oncogene.

SPECIFIC INTERCHROMOSOMAL TRANSlocations are repeatedly found in distinct types of malignancies (*1*). Chromosomal translocations found in lymphoid neoplasms provide the opportunity to identify new putative proto-oncogenes introduced into either the immunoglobulin loci of B cells or the T cell receptor (TCR) loci of T cells. For example, the t(14;18) of follicular B cell lymphoma revealed BCL-2 juxtaposed with the immunoglobulin heavy chain gene (*2*). Transgenic mice established the prospective oncogenic importance of this new gene in malignant lymphomagenesis (*3*). Similarly, the most frequent site of chromosomal aberration in T cell acute lymphoblastic leukemia (T-ALL) represents the α and δ subunits of the TCR at 14q11. Approximately 7% of T-ALLs possess the t(10;14)(q24;q11) (*4*). Rearrangements of the TCR were used to clone breakpoints within chromosome segment 10q24 that clustered around an evolutionarily conserved region (*5, 6*).

A conserved 1.3-kb Pvu II–Bam HI fragment of 10q24 origin (Fig. 1) recognized a transcript of the proposed TCL3 gene in t(10;14) T-ALLs (*6*) (Fig. 2). To identify the normal cellular lineage that expressed TCL3, we hybridized this probe to Northern panels of RNA from human and murine organs. Human liver was the only organ to contain demonstrable TCL3 RNA as did murine liver, attesting to its evolutionary conservation (Fig. 2). While normal liver contained TCL3, the hepatocellular carcinoma line HepG2 did not. TCL3 was not detected in thymus (Fig. 2), repeated thymus samples, resting T cells, or T cells activated with phytohemagglutinin (PHA) or

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