doi:10.1016/j.jmb.2006.02.020 J. Mol. Biol. (2006) 358, 943–962

JMB

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Oligomerisation of the Developmental Regulator Proline Rich Homeodomain (PRH/Hex) is Mediated by a Novel Proline-rich Dimerisation Domain

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Homeodomain proteins regulate multiple developmental pathways by altering gene expression temporally and in a tissue-specific fashion. The Proline Rich Homeodomain protein (PRH/Hex) is a transcription factor and an essential regulator of embryonic development and haematopoiesis. Recent discoveries have implicated self-association as an important feature of transcription factor function. Here, we show using a variety of techniques including gel-filtration, analytical ultracentrifugation, electron microscopy and *in vitro* cross-linking, that purified recombinant PRH is oligomeric and we use *in vivo* cross-linking to confirm that this protein exists as oligomers in cells. This is the first demonstration that a homeodomain protein can oligomerise *in vivo*. Consistent with these findings we show that a fraction of endogenous and exogenous PRH appears as discrete foci within the nucleus and at the nuclear periphery. The N-terminal domain of PRH is involved in the regulation of cell proliferation and transcriptional repression and can make multiple protein–protein interactions. We show that this region of PRH contains a novel proline-rich dimerisation domain that mediates oligomerisation. We propose a model that explains how PRH forms oligomers and we discuss how these oligomers might control transcription.

Keywords: transcription; chromatin; homeodomain; oligomerisation; PRH

Introduction

Transcription factors often bind to specific DNA sequences in order to bring about the regulation of gene expression. In most cases, these proteins appear to function as either homo or hetero-dimers, although in some cases they act as monomers, or as higher-order complexes, such as trimers or tetramers. However, recent work has suggested that some transcription factors form much larger oligomeric assemblies. For instance, the activator protein EBNA-2,[1] the repressor protein TEL,[2] the corepressor proteins ETO[3] and Groucho/TLE,[4] as well as several proteins involved in nucleosome remodelling and the formation of silencing complexes such as RING1[5] and GAGA,[6] all have self-association domains. The ability to form oligomers appears to be a feature of transcriptional silencing mechanisms that involve chromatin compaction (reviewed by Gaston and Jayaraman[7]). For example, the Sir proteins, required for telomeric silencing and the silencing of mating type in yeast, form oligomeric complexes that are thought to bring about transcriptional repression by spreading along chromatin fibres. The ability of PcG and heterochromatin proteins of the HP1 family to self-associate and form hetero-oligomers is also important for the formation and spread of silencing complexes.[5,8] Similarly, members of the Groucho/TLE family of co-repressor proteins form tetramers[4] and can also assemble into larger oligomeric structures.[9] Groucho/TLE proteins that cannot oligomerize fail to repress transcription, demonstrating that in this case oligomerisation is essential for repression.[9,10]

Some transcription factors that are disrupted in leukaemia and cancer are also thought to form

Abbreviations used: PRH, Proline Rich Homeodomain; HDC, PRH Homeodomain and C terminus; PML, Promyelocytic Leukaemia protein; GST, glutathione-*S*-transferase; AD, activation domain; DBD, GAL4 DNA-binding domain.

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oligomers in cells. The repressor protein TEL, for example, is a DNA binding protein of the Ets family that contains an oligomerisation domain known as the SAM domain. This domain is also important for transcriptional repression by TEL[2,11] and the isolated SAM domain can form filaments *in vitro*.[12] In a number of leukaemias oncogenic fusion proteins containing the SAM domain form oligomeric complexes and this implicates the oligomeric SAM domain in cell transformation.[2,13] Another example is the PML protein. PML is a multifunctional protein that inhibits cell proliferation and regulates transcription.[14] Chromosomal translocations that disrupt the PML gene can result in the production of PML fusion proteins that perturb the normal function of PML and disrupt PML nuclear bodies resulting in acute promyelocytic leukaemia. oligomeric PML is the major structural constituent of discrete sub-nuclear particles known as PML nuclear bodies, PODs, nuclear bodies or ND10 domains. PML and PML nuclear bodies are essential for the control of cell proliferation. These structures also play a role in the regulation of transcription and they may be involved in the sequestration and/or processing of transcription factors.[15–19] PML oligomerisation is mediated by a structural domain known as the RING domain,[20,21] which inhibits cell proliferation through a direct interaction with translation factor eIF4E.[22–24] The disruption of PML oligomerisation by viral proteins that bind to the RING domain leads to the loss of PML nuclear bodies during viral infections and to increased cellular proliferation.[25,26]

Homeodomain proteins are transcription factors that generally bind to DNA as monomers,[27,28] homodimers,[29] or heterodimers with other homeodomain proteins[30,31] or DNA-binding proteins from other families.[32] In some cases, the presence of additional protein–protein interaction domains allows the participation of homeodomain proteins in multiprotein complexes. For example, the LIM homeodomain proteins contain a protein–protein interaction domain known as the LIM domain and use this domain to interact with CLIM cofactor proteins. Tetramers consisting of a cofactor dimer and two LIM homeodomain proteins form on DNA.[33] However, to date there is no biochemical evidence to suggest that homeodomain proteins form homo-oligomers in cells.

The Proline Rich Homeodomain (PRH) protein (also known as Hex)[34,35] plays a variety of roles in the control of cell differentiation and cell proliferation. In the adult, PRH functions as a regulator of haematopoiesis.[36–42] PRH inhibits the proliferation of haematopoietic cells of myeloid lineage[39,42] although paradoxically PRH can also function as an oncoprotein in haematopoietic cells of T-cell lineage.[36,38] In addition to its role in haematopoiesis, PRH is involved in the control of many processes in embryonic development including embryonic patterning, formation of head, forebrain, thyroid, liver and heart and development of the vasculature.[43–48] PRH generally represses transcription although it can also activate transcription in some cirumstances.[44,49–52] PRH represses transcription using a number of mechanisms. These include binding to specific DNA sequences,[50] and/or interacting with other proteins, for example, the TATA box binding protein (TBP),[50] the corepressor Groucho/TLE[53] and the activators c-jun[54] and GATA-2.[55] The mechanisms by which PRH activates transcription are less well understood.

The PRH protein contains an N-terminal domain that is 20% proline-rich, a central homeodomain that is essential for binding to DNA, and an acidic C-terminal domain. The PRH N-terminal domain is required for the inhibition of myeloid cell proliferation and cell transformation.[39,42] This region of PRH is also required for the interaction of PRH with both PML and eIF4e in PML nuclear bodies.[39,56] Our recent work has shown that the PRH N-terminal domain represses transcription when attached to a heterologous DNA binding domain[50] and that this is due in part to the recruitment of Groucho/TLE to DNA.[53] Here, we show that PRH can exist in an oligomeric form in cells. We show that the N-terminal domain of PRH contains a novel proline-rich dimerisation domain and that this region of PRH mediates oligomerisation. We suggest a model for the oligomerisation of the full-length PRH protein and we discuss these results with reference to the regulation of transcription and cell proliferation.

Results

PRH forms nuclear foci

PRH is expressed in several cell types in the embryo and in the adult. We have shown previously that PRH is highly expressed in the leukaemic K562 myeloid cell line and that PRH can repress transcription in these cells.[42,53,57] K562 cells derive from a patient with chronic myeloid leukaemia (CML) in blast crisis and in culture they can give rise to undifferentiated blasts and to smaller more differentiated myeloid cells with little or no cytoplasm.[58] Thus K562 cells are not a uniform cell population. Immunofluorescent staining with a mouse polyclonal antibody raised against the N-terminal domain of PRH shows that K562 cells have both diffuse nuclear PRH staining and punctate nuclear PRH staining and that in some cells punctate staining of the nuclear periphery is also present (Figure 1(a) and (b)). Some cells that have large amounts of cytoplasm also appear to show, in addition, strong cytoplasmic staining (Figure 1(c)). These cells are K562 blasts and are present in lower number in the total cell population.

To confirm these findings we determined the subcellular localisation of a Myc-tagged PRH protein (see Materials and Methods). K562 cells were transiently transfected with a plasmid expressing Myc-tagged PRH and immunofluorescent staining was carried out using the Myc9E10