Cell Lineage Specificity of Chromatin Configuration and Gene Expression in Haemopoietic Progenitor Cells

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Haemopoietic stem cells have the potential to activate up to eight distinct cell lineage specific genetic programmes [1]. The mechanisms of cell lineage choice or commitment are fundamental to developmental biology in general and are beginning to be unravelled at least in invertebrates (e.g., Drosophila, Nematodes, slime moulds). Transacting DNA binding proteins that directly or indirectly regulate gene transcription are central players in the game [2], as are inductive cellular interactions [3]. In haemopoietic differentiation, it is clear that selective progenitor-stromal cell interactions involving both adhesive and growth factor recognition [4–7] are playing a role in early decision-making but the basic mechanisms whereby uni-lineage adoption is made are still obscure. A sample scheme might incorporate the following possibilities:

1. That when mesodermal cells become committed to blood cell formation [1] as opposed to say, muscle, then this specification must be reflected in inheritable changes in DNA structure and should logically include changes in genes that are functionally coupled to the individual lymphoid and myeloid lineage programmes. These genes could encode transcription factors, growth factor receptors, adhesion receptors and other functionally important molecules.
2. Alterations in genes that register pan-haemopoietic commitment plus potentiality for specific haemopoietic lineages could involve active transcription and/or alterations in chromatin structure. It is known that active genes are preferentially sensitive to DNAse I and that transcriptional control regions are hypersensitive [8–10]. Accessibility of chromatin to DNAse I is necessary but not sufficient for transcription and may provide therefore a convenient marker for genes that have become primed for activation.
3. That lineage programmes are expressed as a coordinated cascade of transcriptional control and the commitment process itself involves only a small number of genes, i.e. those that are expressed earliest in the developmental programme. Recent studies on muscle cell differentiation have provided an instructive precedent, indicating that single genes coding for regulatory (DNA binding) proteins can initiate a full programme of striated muscle lineage specific gene expression [11, 12].

With this background, we have asked whether any of the known haemopoietic lineage-associated genes are either transcriptionally active or have DNAse I hypersensitive sites in multi-potential cells. Immunoglobulin heavy chain and T-cell receptor (γ, β) genes were obvious choices as they have (sterile) transcripts (prior to gene rearrangement) in the earliest identifiable B or T lymphocyte percursors and their genomic structure is known in considerable detail [13, 14].

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We first screened a series of human lymphoid and myeloid leukaemia cell lines and normal tissue for DNAse I hypersensitivity of the IgH enhancer region, methylation of Hha I sites flanking this region and sterile mRNA. These results have been published in detail elsewhere [15].

As tested by DNAse I hypersensitivity, the chromatin structure of the IgH enhancer region in human B-cell precursor cell lines was in an open or accessible conformation. All T-cell lines, with either germline or rearranged IgH genes, were also hypersensitive to DNAse I but in contrast to B-cell precursors showed no detectable Cμ expression. Normal thymocytes similarly had a hypersensitive IgH enhancer site. In contrast to lymphoid cells, all myeloid cell lines tested, as well as normal granulocytes, were not DNAse I hypersensitive and did not express Cμ. Two Hha I restriction sites on either side of the IgH enhancer were not methylated in all Cμ-expressing lines but methylated in non-expressing cell lines. A putative lympho-myeloid progenitor cell line KG1 [16], although having a germline configuration of Ig genes, produced Cμ transcripts (and TCR γ mRNA) and was hypersensitive to DNAse I in the IgH enhancer region. After induction of myeloid differentiation the Ig enhancer region of KG1 cells is no longer hypersensitive or transcriptionally active. These results show that an open chromatin structure around the heavy chain enhancer is necessary but insufficient for initiating transcription from unrearranged IgH genes and further suggests that this region may be in an open or accessible configuration prior to lineage commitment and close following adoption of the myeloid lineage.

To pursue this possibility further, we have performed similar analyses in murine IL3-dependent cell lines established from long-term bone marrow culture [17, 18]. These lines have the considerable advantage over human cell lines (such as KG1) that they retain multilineage differentiation potential in vivo and in vitro, are nonleukaemogenic and have a normal diploid karyotype.

Four independent cell lines have been tested with similar results; we show here results with one line, A4. The cells maintained in medium supplemented with IL3 have a DNAse I hypersensitive IgH enhancer site as revealed by the presence of a 1.8 kb DNAse I digest product (EHS in Fig. 1, lane 2). There is however no stable μ mRNA detectable. TCR γ but not β genes are transcribed and in vitro hybridization analysis with a ^{35}S-DNA TCR γ probe indicates that $>95\%$ of A4 cells contain TCR γ mRNA. When A4 cells are induced to differentiate by removing IL3 and placing the cells in contact with either normal bone marrow stroma or 3T3 fibroblasts, then they differentiate into various types of myeloid cells (but predominantly granulocytes). Analysis of the differentiated progeny of A4 cells reveals that the IgH enhancer region is now resistant to DNAse I (i.e. closed; Fig. 1) and no TCR γ mRNA is detectable (Fig. 2) compared with an actin mRNA control.

We interpret these results to indicate that normal primitive myeloid progenitors have active or "primed" lymphoid genes but that these are closed down when definitive myeloid differentiation occurs. Since we also find that primitive embryonic stem cells (ES cells; [19]) and the primitive pan-mesodermal cell line 10T1/2 [20] have a DNAse I resistant IgH enhancer region and no TCR γ transcripts, we consider it likely that these features are characteristic of haemopoietic stem cells.

Experiments are in progress with other haemopoietic genes that are expressed very early in haemopoietic differentiation, e.g. CD3, CD2, CD19, λ 5, β-spectrin, CD33, and MPO, to see if they are transcriptionally active or are primed with DNAse I hypersensitive enhancer regions.

One speculative interpretation of these data is that haemopoietic stem cells register their multi-lineage potential by activating a small set of regulatory genes

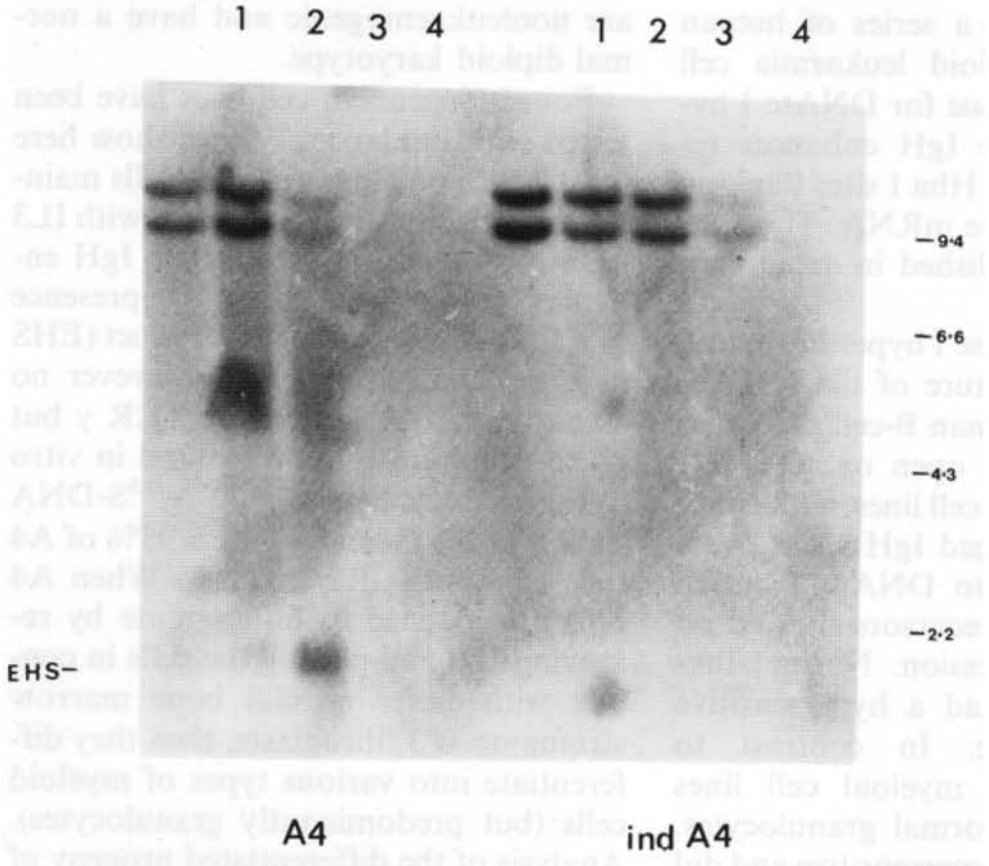

Fig. 1. DNAse I hypersensitive site analysis of the μ gene in myeloid-induced and non-induced A4 cells. *Lane 0:* nuclei incubated without added DNAse I. *Lanes 1–4:* nuclei incubated with 1.2, 1.8, 2.7 and 4 μg/ml DNAse I respectively (each lane contains 10 μg DNA restricted with BamHI and hybridized to a JH-enhancer intron probe). EHS, enhancer hypersensitive site; molecular weight markers are given in kb

whose protein products confer a DNAse I hypersensitive configuration on genes that play a pivotal and possibly initiating role in uni-lineage commitment. Interaction of such cells with appropriate stroma-associated environmental ligands [5, 6] might then selectively up-regulate particular transcription factor(s) and so initiate a cascade of selective gene expression for T cells or granulocytes, etc. Adoption of one lineage also then involves closing down the availability of other previously accessible lineage restricted genes.

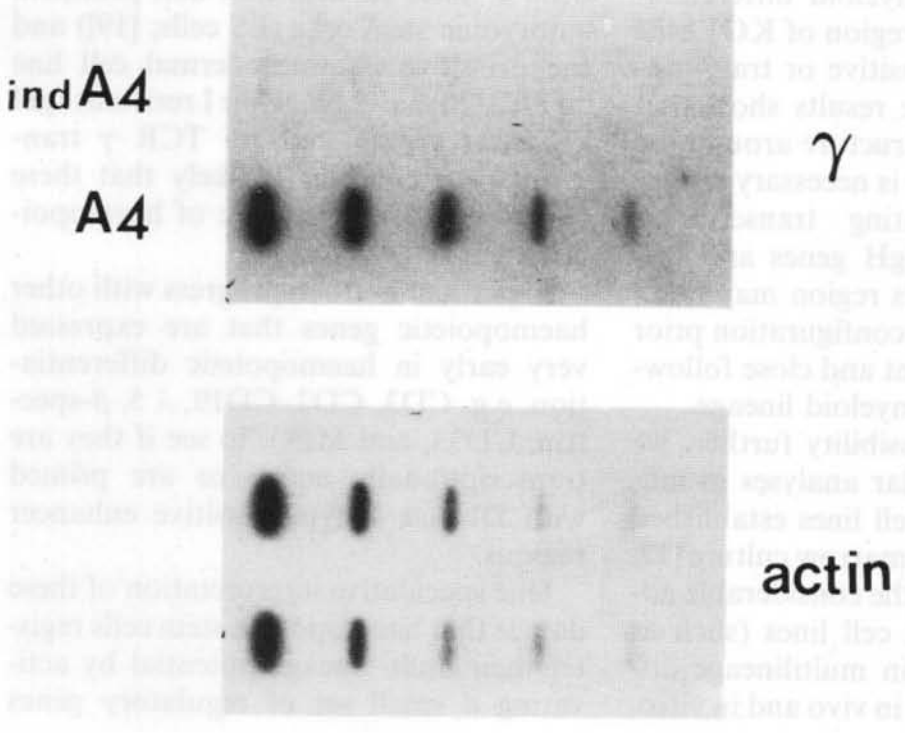

Fig. 2. RNA slot blot analysis of cytoplasmic RNA extracted from induced and non-induced A4 cells. Each lane contains samples of 2, 1.0, 0.5, 0.25 and 0.125 μg total cellular RNA, hybridized to murine TCR γ and actin probes respectively

Clearly many more experiments are required to verify these ideas. In particular, we need to establish that the results obtained to date are not unique to the rearranging lymphoid genes or to the cell lines used.

Finally, these data have potentially important implications for an understanding of lineage specific gene expression in human leukaemia cells. A proportion of acute leukaemias (5%–10%) display multilineage gene expression in individual blast cells [21]. It was suggested that this phenotypic pattern reflected either (a) infidelity of gene expression or genetic misprogramming arising as a direct consequence of gene rearrangements in leukaemia, or (b) an origin of such leukaemias in multi-lineage progenitor cells with effective maturation arrest in a proliferating mode such that their intrinsic capacity to activate early components of the lineage specific programme is revealed. This latter interpretation accords with the data we report in this paper and elsewhere [15]. The two interpretations are not exclusive however; certainly it is possible that activated or mutated proto-oncogenes could have profound effects on the regulation of lineage specific gene programmes [22].

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