1. PUBLISHABLE SUMMARY

Murine Embryonic Stem (ES) cells are derived from pre-implantation mouse embryos and can be maintained indefinitely in culture without losing the ability to differentiate in all cell types constituting the adult body, a propriety defined "pluripotency". As such they constitute an invaluable model to study the properties of the related human ES cells, a potential source of material for future therapeutic approaches aiming at replacing damaged cells and reconstitute functional tissues in patients.

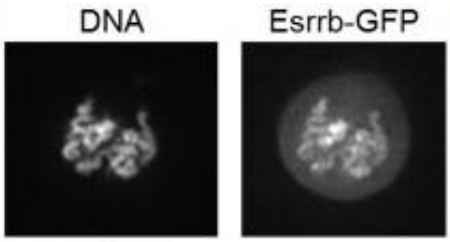

Figure 1: Live-imaging of Esrrb mitotic localisation.

The identity of a cell type is determined by the particular combination of genes that it expresses. In turn, transcription factors are the proteins responsible of controlling such identity and exert their function by physically binding to stretches of DNA displaying a particular sequence of bases, and recruiting the transcriptional machinery in proximity of the genes to be expressed. ES cells pluripotent identity depends on the activity of a relatively well characterised group of transcription factors, among which central are Oct4 and Sox2. A number of other ancillary factors confers both robustness and flexibility to the ES cell regulatory network, allowing ES cells to rapidly proliferate perpetuating their identity, a process known as self-renewal: among these is Esrrb. The self-renewal ability of ES cells is key should we want to generate sufficient amount of undifferentiated cells for therapeutic applications, and the study of the factor governing this process is of undisputable relevance.

The ability of ES cells to indefinitely transmit the activity of the pluripotency network across cell division is remarkable, since mitosis is thought to represent an obstacle to the continuity of control by transcription factors. In order to divide cells need to duplicate and compact their DNA, before allocating a copy to each daughter cell. Compaction results in the displacement of most transcription factors from DNA, posing the problem of re-instating the correct regulatory architectures in newly formed cells. Direct chemical modifications of the DNA, or the proteins stably associated with it, are thought to mark genes that need to be expressed or kept silent in a particular cell type. Such modifications are inherited through cell division and are thought to guide the re-establishment of transcription factor control in daughter cells. ES cells constitute an interesting exception in this regard, as abrogation of the activities responsible for deposing such marks is largely inconsequential. Combined to their rapid proliferation cycles, this makes of ES cells a privileged model to study the importance of additional mechanism for transmitting cell identity. One of such mechanism may be based on the ability of certain transcription factors to remain bound to DNA during cell division, a process known as "mitotic bookmarking".

We set out to explore this possibility and created a cell line in which Esrrb is marked by fusion to a fluorescent protein. Microscopy on live cells clearly shows that Esrrb globally retain binding to DNA during division (**Fig. 1**). We further showed, by use of a technique that allows to map all regions of DNA to which a protein is bound, that in mitosis Esrrb specifically retains binding to a functionally relevant subset of the regions it occupies before division (**Fig. 2**), in proximity of genes of particular importance for ES cell identity. By mutating the domain of the protein responsible for DNA binding, we demonstrate that mitotic localisation is driven by the ability of Esrrb to recognise specific DNA sequences. Similarly, manipulating the DNA of ES cells to introduce synthetic copies of such sequences results in Esrrb recruitment during division. Furthermore, we directly imaged the dynamics of Esrrb interaction with DNA and showed that Esrrb continuously binds and detaches from it, rather that statically occupying certain positions throughout division: this mode of action is similar to what observed in non-mitotic cells, suggesting that bookmarking transcription factors may exert their regulatory control in canonical ways during division. Finally, we constructed an ES cell line in which Esrrb protein can be rapidly depleted in response to the addition of a chemical compound to the culture medium. The same cell line was engineered to express a fluorescent protein that is degraded during each mitosis, allowing to identify cells that have just divided. By depleting Esrrb before division and measuring the effect on the

expression of the genes it controls in newly formed daughter cells, we were able to show that Esrrb mitotic binding contributes to the reactivation of the correct set of genes after division, providing a mean to ensure stable transmission of cell identity. We recently extended our observations to the mitotic behaviour of Sox2 and Oct4, showing that while the first globally localises on DNA during mitosis, retaining weak binding to specific regions of functional significance, the second is vastly displaced from the positions it occupies before division. Esrrb and Sox2 binding in mitosis partially overlaps, defining a core set of DNA regions of crucial importance for the transmission of ES cell identity.

The results of our study have a clear impact on the current knowledge of how regulatory control over transcription, and thus cell identity, is inherited through cell division. Our conclusions extend generally to cell types other than pluripotent ES cells. In conjunction with independent recent reports, a picture is forming in which mitosis is no more perceived as entailing a complete collapse of regulatory architectures. We contributed highlighting how mitotic DNA, although compacted, maintain a remarkable accessibility to transcription and regulatory factors.

Having uncovered a completely novel aspect of how pluripotent ES cells maintain indefinitely their identity in culture, we foresee the results of our study having implications in protocols aiming at expanding undifferentiated progenitors or directing their differentiation in cell types of therapeutic relevance. Thus, our study is of interest to the wider medical community, and to patients groups or organisations. The publication of our study inspired novel research and resulted in a series of reports expanding our conclusions. The general interest in mitotic bookmarking, and specifically its implications for the field of stem cell biology and reprogramming, is likely to expand in the future, calling for an increased attention of funding bodies on this topic.

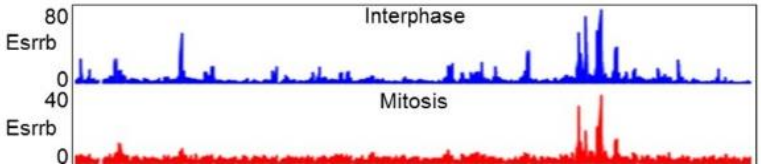

Figure 2: ChIP-seq profile of Esrrb DNA binding in mitosis and interphase