cytes, and this is currently the favored procedure for generating DCs for immunotherapy. Despite this central role for GM-CSF in culture systems, mice deficient in either GM-CSF or its receptor still produce DCs (*13*). In addition, enhancing GM-CSF levels in mice produces only modest increases in DC numbers, in contrast to another cytokine, Flt-3 ligand (*13, 14*). In part, this reflects the existence of other DC lineages, less dependent on GM-CSF (*15*), although it may also indicate that other stimuli can induce differentiation of even the macrophage-related DCs.

The culture system of Randolph *et al.* does not require exogenous cytokines. Rather, two different nonhormonal stimuli that can drive monocytes to develop into DCs were delineated, phagocytosis and transendothelial transport. In the model of Randolph *et al.*, monocytes first cross a layer of endothelial cells and lodge in a collagen matrix, mimicking entry of monocytes into tissues from the bloodstream (see figure at left). A proportion of these monocyte-derived cells then "reverse transmigrate," mimicking migration of DCs out of the tissues into lymph. Those that remain in the "tissue" become macrophages. Those that reverse transmigrate become DCs. The extent of this differentiation and transmigration is greatly enhanced if the cells are undergoing active phagocytosis of foreign particulate material in the collagen matrix. This fits with evidence that phagocytosis of bacteria provides a strong stimulus for DC maturation (*16*). The new and intriguing possibility is that reverse transmigration across an endothelial barrier provides additional signals that induce or enhance differentiation into DCs.

The factors that control movement across the endothelial barrier include chemokines, adhesion molecules, and, surprisingly, the p-glycoprotein (MDR-1) on the DC surface (*17, 18*). But does any part of the transmigration process itself promote DC development, or does the endothelium simply act as a filter, allowing egress of DC-committed cells but retaining in the tissue macrophage-committed cells? And if transmigration induces DC differentiation, are the signals fundamentally different from those revealed by the earlier cytokine-driven cultures? The endothelial cells themselves may be the source of GM-CSF and proinflammatory cytokines, a more efficient presentation by this route accounting for the rapid generation of DCs.

Finally, is a single type of uncommitted blood monocyte driven by environmental factors to become either a macrophage or a DC? The alternative is that blood monocytes are a mixture of cells of predetermined developmental disposition, the endothelial culture system selectively maturing the DC committed precursors, rather than determining DC or macrophage commitment. There is evidence that early myeloid progenitor blasts can produce distinct precursor cells, some with a capacity to form DCs when appropriately stimulated, but others committed exclusively to macrophage production (*19*). It may be possible to answer this question by recycling partially differentiated cells through an endothelial culture system a second time.

DCs have a complex life history. By modeling just one part of DC physiology in culture, Randolph *et al.* have provided unexpected insights into an important developmental decision point. If some of the basic questions still remain, we now have available a new and very useful culture model to help answer them.

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PERSPECTIVES: CHROMOSOME STRUCTURE

How to Compact DNA

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During cell division (mitosis), the chromosomes must be condensed into discrete, compact bodies. For each chromosome, this means packing about 4 cm of DNA into a rod 10 μm long and 1 μm in diameter. In this form, the chromosomes can be separated into two separate sets without entangling or breaking them. For the rest of the cell division cycle (interphase), the chromosomes are much more diffuse structures, long strands that allow access to the DNA by enzymes that replicate DNA and transcribe it into messenger RNA. A report in this issue on page 487 reveals an important insight into how progress through the cell cycle regulates the packing of DNA into chromosomes (*1*).

Which proteins drive these enormous changes in chromosome architecture that occur as cells enter mitosis? How are they regulated by the biochemical oscillator that drives the cell division cycle? Early attempts to study this problem focused on the histone proteins, around which the chromosomal DNA is wrapped and which are the most abundant of the chromosomal proteins. There are major changes in the phosphorylation of histones as cells enter mitosis (*2*), and one protein kinase that performs this phosphorylation (the complex of Cdc2 and cyclin B) is the principal biochemical activity that induce mitosis (*3*). Despite this correlation, however, the importance of histone phosphorylation in mitotic chromosome condensation is unclear.

Hirano and his colleagues have taken an alternative approach to understanding chromosome condensation that exploits the early cell cycles of fertilized frog eggs. Two aspects of these cell cycles make them attractive: First, there is little transcription, so differences between the proteins associated with interphase and mitotic chromosomes are likely to reflect the different structural organization of the two types of chromosomes rather than different transcriptional states; second, the entire cell cycle occurs in extracts made from eggs. In the first experiments, the authors added sperm nuclei to interphase or mitotic extracts, recovered the decondensed or condensed chromosomes, and examined the proteins that were associated with them (*4*). This approach identified two large proteins, XCAP-C and XCAP-E, that were specifically associated with mitotic chromosomes and that contained a long coiled-coil domain and a region with strong homology to other proteins that hydrolyze adenosine triphosphate (ATP). Depleting XCAP-C or -E from the egg extracts prevented mitotic chromosome condensation. An even more interesting result is that inactivating the proteins in extracts that were already in mitosis led to chromosome decondensation, suggesting that con-

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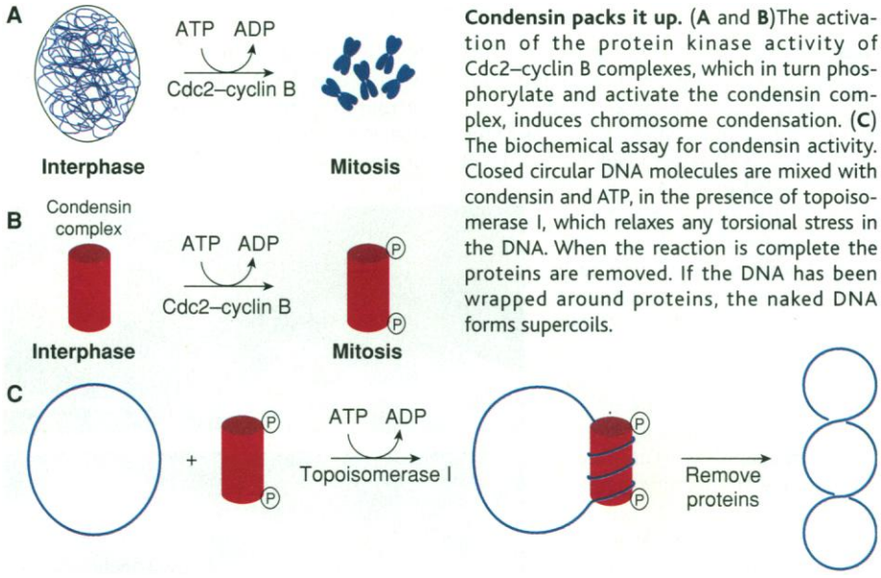
densation is a dynamic state that must be continuously maintained, and that these proteins contribute to the rapid reorganization of chromosome structure that occurs as cells enter mitosis. This idea is strengthened by the finding that XCAP-C and -E are homologous to the Smc1 protein of budding yeast, which is required for accurate chromosome segregation during mitosis (*5*). Members of this protein family are called SMC proteins, and the closest relatives of XCAP-C and -E are involved in chromosome condensation in fission and budding yeasts. Other family members control a wide range of chromosome behavior, including dosage compensation in nematodes (the phenomena that allows the one X chromosome of a male cell to make as much messenger RNA as the two X chromosomes of a female cell), the linkage between the two sister chromosomes that are the product of DNA replication, the repair of DNA damage, and bacterial chromosome segregation (*6, 7*).

XCAP-C and XCAP-E form a complex with three other proteins, dubbed the condensin complex, which can be isolated from both interphase and mitotic cells (see figure). The complex isolated from mitotic extracts of frog eggs can use the energy of ATP hydrolysis to wrap DNA around itself, and when this reaction is performed in the presence of a topoisomerase, the topology of DNA molecules is altered by introduction of positive supercoils into a circular piece of DNA (*8*). A homologous complex from fission yeast can catalyze the rewinding of two unpaired DNA strands into a double helix (*9*). Neither of these activities tells us how these complexes can condense chromosomes, but they do suggest that ATP-driven changes in DNA topology and DNA-protein interactions are likely to be critical for the rapid chromosome condensation and decondensation that occurs as cells enter and leave mitosis.

The latest paper from Hirano's laboratory deals with how the cell cycle machinery regulates chromosome condensation (*1*). Comparison of the condensin complex from interphase and mitotic extracts reveals that three of its subunits become phosphorylated in the mitotic extract and that only the mitotic form of the complex has the ability to supercoil DNA (see figure). Depleting the Cdc2–cyclin B complex from a mitotic extract blocks phosphorylation of the condensin complex, chromosome condensation in extracts, and the supercoiling activity of the condensin complex that has been purified from these extracts. These effects are likely to reflect direct phosphorylation of the condensin complex by Cdc2–cyclin B, because incubating the interphase complex with Cdc2–cyclin B phosphorylates it and activates its DNA supercoiling activity.

Now that the composition and regulation of the condensin complex is on firm ground, the major challenge is to understand how this group of proteins induces chromosome condensation during mitosis. Is the activation of the condensin complex sufficient to induce chromosome condensation, or do other modifications, such as histone phosphorylation, also participate? What are the structural changes in the interactions of DNA, histones, and other proteins that compact the chromosomes during condensation? The jump from the reaction of purified condensins with naked DNA to chromosome condensation in cells and crude extracts is the largest gap in our knowledge. Can pure condensins compact chromatin that has been reconstituted from DNA and pure histones? If not, what other proteins must be present?



Condensin packs it up. (**A** and **B**)The activation of the protein kinase activity of Cdc2–cyclin B complexes, which in turn phosphorylate and activate the condensin complex, induces chromosome condensation. (**C**) The biochemical assay for condensin activity. Closed circular DNA molecules are mixed with condensin and ATP, in the presence of topoisomerase I, which relaxes any torsional stress in the DNA. When the reaction is complete the proteins are removed. If the DNA has been wrapped around proteins, the naked DNA forms supercoils.

One of the most important advances in our understanding of chromosomes has been the appreciation that chromosome structure is almost as important as DNA sequence for determining how chromosomes behave. The same gene can have different levels of activity at different chromosomal positions within the same cell, and in some multicellular eukaryotes important chromosomal features, such as the centromere, are not defined by specific DNA sequences (*10*). Such features may reflect regional differences in condensation between different parts of the chromosomes in interphase cells. Are these differences the result of recruiting mitotic condensins to specific regions of interphase chromosomes, or do they reflect the activity of related complexes that are specific for particular regions of interphase chromosomes? The most remarkable form of interphase condensation occurs in female mammals, where the problem of dosage compensation is solved by X inactivation: the condensation and transcriptional silencing of one of the two X chromosomes throughout the cell cycle. In addition, substantial regions of the chromosomes form heterochromatin, more highly condensed regions of the chromosomes that are maintained in this state throughout interphase. Do molecules that are required for X-inactivation and heterochromatin formation recruit condensins or condensin-like complexes to specific regions of chromosomes? A precedent for one complex performing two functions in this way occurs in nematodes, where the homolog of XCAP-E acts specifically on the X chromosome during dosage compensation and generally on all the chromosomes in mitosis (*11*). The next few years promise molecular answers to questions that have been fascinating biologists ever since Boveri first suggested that the number and type of chromosomes determined the fates of cells (*12*).

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