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Editorial

A closer look at the cell cycle

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Features which characterize malignant tumour cells include their disturbed proliferative activity as well as their genetic instability, the latter often leading to aneuploidy. Both characteristics are correlated with biological behaviour; particularly unfavourable courses are found in highly proliferative and aneuploid tumours (Baak 1991). Genetic instability may represent an important factor in tumour progression (Nowell 1976).

Over the last 10 years numerous genes have been identified whose mutation leads to autonomous proliferation and which most probably contribute to tumorigenesis (Bishop 1991). Many of these are proto-oncogenes, their protein products acting as links in the chain of cellular signal transduction, which converts an extracellular, mitogenic signal into an intracellular one (Cantley et al. 1991). The secondary processes finally initiated by growth signals, such as DNA replication and cell division, appeared at first to be of minor pathogenetic importance in the development of neoplasms (Marx 1991) but recent results indicate that not only do the structures involved in the induction of proliferation contribute to autonomous growth, but that the cellular apparatus which manages DNA replication and cell division is also important.

As an example, the cyclin A gene in hepatitis B virus-associated liver cell carcinoma is affected by insertional mutagenesis (Wang J et al. 1990). In cells transformed by adenovirus the cyclin A protein complexes with the oncogenic E1A protein (Pines and Hunter 1990). As a consequence of translocation and juxtaposition in parathyroid neoplasms the *PRAD1* gene comes under the influence of the parathormone gene enhancer, and is overexpressed; *PRAD1* codes for cyclin D (Motokura et al. 1991). The tumour suppressor genes Rb (retinoblastoma) and *p53* are directly involved in the regulation of the cell cycle. *Rb* complexes with the E2F transcription factor, which controls activation of S-phase-specific genes (e.g. DNA polymerase α; Weintraub et al. 1992) and *p53*, which is commonly inactivated or mutated in tumours, regulates the initiation of the S phase and helps to ensure the replication of intact DNA only (Diller et al. 1990; Kastan et al. 1991). These intriguing research results provide the prospect of a better understanding of the pathogenesis of neoplasms, as they offer an explanation for the disturbed proliferative behaviour and for the characteristic genetic instability of tumour cells. It is clear that we need a more detailed understanding of those processes controlled by the cell cycle, such as DNA replication and cell division. In recent years considerable progress has been achieved in the understanding of the cellular mechanisms and structures involved in replication.

By analysis of temperature-sensitive deficiency mutants in fungi, numerous genes involved in the regulation of the eukaryotic cell cycle have been identified (Lewin 1990). Because of the potential lethality of mutations within cell cycle genes it was important to provide a rescue mechanism for the affected cells that otherwise would not have been clonable. By selecting for mutations that caused loss of functions only below a certain temperature it was possible to overcome this problem since shifting to the permissive temperature enabled normal growth. From these studies two different types of regulatory proteins emerged. The first group consists of kinases and their antagonist phosphatases that activate or de-activate other proteins in a cell-cycle-specific fashion by adding or removing phosphate residues. The second group comprises proteins that are produced and decay in a cell-cycle-phase specific manner; these are called cyclins.

Mutations of the *cdc2* gene led to the discovery of the $p34^{cdc2}$ kinase that fulfils central functions at the G1-S transition and at the onset of the M phase (Nurse 1990). Substrates of $p34^{cdc2}$ serine/threonine kinase are, amongst others, nuclear lamin B, histone H1, $pp60^{c\text{-}src}$, nucleolin and the Rb gene product, which may point to a possible role in diverse cell-cycle-dependent processes such as disintegration of the nuclear membrane, chromosome condensation, rearrangement of the cytoskeleton and lysis of the nucleolus (Moreno and Nurse

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1990). Regulation of protein kinase $p34^{cdc2}$ activity is again achieved by phosphorylation and dephosphorylation of a tyrosine residue. In order to become active the kinase has to be dephosphorylated at this site. The genes *wee1* and *mik1* (inhibiting) and *cdc25* (activating) are involved in the control of this regulatory mechanism (Enoch and Nurse 1991). Mutants possessing defects in the inhibiting genes undergo premature entry into the M phase, before the completion of chromosome replication (Enoch and Nurse 1991).

Together with another functionally relevant subunit, cyclin B, $p34^{cdc2}$ forms the "maturation-promoting factor" (Draetta et al. 1988). Cyclins are proteins expressed during certain phases of the cell cycle and then rapidly broken down at the onset of another cell cycle phase (Glotzer et al. 1991). In mammalian cells five groups of cyclins (A–E) are distinguished and are expressed at the transition from G2 to M phase (A, B), in the S phase (A) or during the G1 phase (C–E; Hunter and Pines 1991). During the G1 phase the D cyclins complex with cyclin-dependent kinases, which show a relation to $p34^{cdc2}$. Several cyclins, whose expression is in part tissue-specific (*CYL 1* and *3*; Matsushime et al. 1991), appear to be responsible for the coordinated transition into the S phase.

The sequence of phases in the cell cycle requires that each succeeding step be carried out only *after* completion of the previous step. In order to fulfil this mandatory requirement the cycling cell passes so-called checkpoints ensuring that the previous phase has been perfectly accomplished before transition into the next phase is initiated. An example of a checkpoint is the occurrence of $p34^{cdc2}$ activation after completion of DNA replication. Additional genes are involved in this control mechanism before entry into the S phase, such as the *rad9* gene in fungi and the *RCC1* gene, also occurring in mammals, which complexes with a nuclear protein resembling *ras* (Bischoff and Ponstingl 1991). Less is known about the "checkpoints" at the G1-S transition. In this context, the product of the tumour suppressor gene *p53* may play an important role (Kastan et al. 1991). Several genes specifically expressed in the S phase, such as thymidine kinase and DNA polymerase α, are activated by the transcription factor E2F. This factor is involved in the regulation of the G1-S transition by complexing with other proteins, such as Rb protein (Mudryj et al. 1991; Weintraub et al. 1992). It remains to be established whether aneuploid tumours exhibit defects in these elaborate control mechanisms that guarantee the regular DNA content in the normal cell. The regulatory mechanisms discovered so far provide a number of potential causes of aneuploidy.

Apart from these advances in the identification of regulatory molecules in the cell cycle, considerable progress has also been made in recent years in the identification of "functional" and "structural" proteins, whose activity and modification comprise the dynamics of the cell cycle processes. DNA replication begins at one or more specific sites (the "origins") to which a multi-protein complex binds selectively, bringing about the unfolding/opening of the double strand by a helicase (Bell and Stillman 1992). Replication and elongation of DNA then follows involving the replication factor C, the polymerases α and δ with cofactor PCNA, as well as a DNA primase (Stillman 1989; Wang 1991). Topoisomerase II assists in chromosome condensation with the specific role of eliminating recombinations (Wang J et al. 1990). The present state of knowledge on DNA replication and chromosome condensation does not explain how genetic instability in tumours with frequent aneuploidy, chromosomal translocations, and amplification of specific genes (a rare occurrence in non-neoplastic cells; Wright et al. 1990) comes about.

Similarly, the role of the nuclear scaffold in replication and tumour pathogenesis is unknown to a large extent, mainly due to methodological difficulties in the isolation of the elements of the nuclear skeleton (Pienta et al. 1989; Cook 1991). Cell division requires disintegration of the nuclear membrane, which disintegrates into vesicular elements following phosphorylation by $p34^{cdc2}$, and is reversibly bound to chromosomal chromatin (Peter et al. 1990). Using antinuclear antibodies from autoimmune sera, the elements of the kinetochore complex at the chromosome centromeres, upon which the connection between the chromosomes and the mitotic spindle is made, have been defined (CENAP-A-E; Bernat et al. 1991). Microtubules and dynein are involved in chromosome segregation, as was shown with monoclonal antibodies against dynein (Pfarr et al. 1990; Steuer et al. 1990). Contributing to the construction of the spindle apparatus are nuclear proteins, e.g. NuMA, centrophilin, mitotin (Todorov et al. 1992) and the 210 kDa SPN protein, defined by a monoclonal antibody (Kallajoki et al. 1991). Changes specific to the cell cycle also affect the nucleolus; however, very little information is available on this.

A series of nucleolar proteins, expressed in a cell-cycle-dependent fashion, have been defined by monoclonal antibodies and autoimmune sera (Reddy et al. 1989; Gorczyca et al. 1992). The Ki-67 antigen displays an accentuated nucleolar location; its function is not yet known (Gerdes et al. 1991). During M. phase it is located on the chromosomes. Nucleolar proteins may be located on the surface of chromosomes during mitosis. However, it is still unclear whether this location corresponds to a function in chromosome condensation or reflects deposition and division during mitosis (Gautier et al. 1992). It has been suggested that the Ki-67 antigen is an integral part of the nuclear scaffold (Verheijen et al. 1989). We have developed a number of monoclonal antibodies recognizing the Ki-67 antigen as well as a yet unknown proliferation-associated nuclear antigen, displaying identical spatial and temporal distribution in proliferating cells (Kreipe et al. 1993). It seems likely that immunohistochemical detection of proliferating cells will contribute to the grading of tumours in the future. Both the labelling and assessment of abnormally proliferating cells and the pathogenesis of disturbed DNA replication await further analysis in an interesting and expanding research field in pathology.

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References

Baak JPA (1991) Manual of quantitative pathology in cancer diagnosis and prognosis. Springer, Berlin

Bell SP, Stillman B (1992) ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. Nature 357:128–134

Bernat RL, Delannoy MR, Rothfield NF, Earnshaw WC (1991) Disruption of centromere assembly during interphase inhibits kinetochore morphogenesis and function in mitosis. Cell 66:1229–1238

Bischoff FR, Ponstingl H (1991) Mitotic regulator protein RCC1 is complexed with a nuclear *ras*-related polypeptide. Proc Natl Acad Sci USA 88:10830–10834

Bishop JM (1991) Molecular themes in oncogenesis. Cell 64:235–248

Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S (1991) Oncogenes and signal transduction. Cell 64:281–302

Cook PR (1991) The nucleoskeleton and the topology of replication. Cell 66:627–635

Diller L, Kassel J, Nelson CE, Gryka MA, Litwak G, Gebhardt M, Bressac B, Ozturk M, Baker SJ, Vogelstein B, Friend SH (1990) p53 functions as a cell cycle control protein in osteosarcomas. Mol Cell Biol 10:5772–5781

Draetta G, Piwnica-Worms H, Morrison D, Druker B, Roberts T, Beach D (1988) Human cdc2 protein kinase is a major cell-cycle regulated tyrosine kinase substrate. Nature 336:738–744

Enoch T, Nurse P (1991) Coupling M phase and S phase: controls maintaining the dependence of mitosis on chromosome replication. Cell 65:921–923

Gautier T, Dauphin-Villemant C, Andre C, Masson C, Arnoult J, Hernandez-Verdun D (1992) Identification and characterization of a new set of nucleolar ribonucleoproteins which line the chromosomes during mitosis. Exp Cell Res 200:5–15

Gerdes J, Li L, Schlueter C, Duchrow M, Wohlenberg C, Gerlach C, Stahmer I, Kloth S, Brandt E, Flad HD (1991) Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol 138:867–873

Glotzer M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. Nature 349:132–138

Gorczyca W, Bruno S, Melamed MR, Darzynkiewicz Z (1992) Cell cycle-related expression of p120 nucleolar antigen in normal human lymphocytes and in cells of HL-60 and MOLT-4 leukemic lines: effects of methotrexate, camptothecin, and teniposide. Cancer Res 52:3491–3494

Hunter T, Pines J (1991) Cyclins and Cancer. Cell 66:1071–1074

Kallajoki M, Weber K, Osborn M (1991) A 210 kDa nuclear matrix protein is a functional part of the mitotic spindle; a microinjection study using SPN monoclonal antibodies. EMBO J 10:3351–3362

Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51:6304–6311

Kreipe H, Heidebrecht HJ, Hansen S, Röhlk W, Kubbies M, Wacker HH, Tiemann M, Radzun HJ, Parwaresch MR (1993) A new proliferation-associated nuclear antigen detectable in paraffin-embedded tissues by the monoclonal antibody Ki-S1. Am J Pathol 142:3–10

Lewin B (1990) Driving the cell cycle: M phase kinase, its partners, and substrates. Cell 61:743–752

Marx J (1991) The cell cycle: spinning farther afield. Science 252:1490–1492

Matsushime H, Roussel MF, Ashmun RA, Sherr CJ (1991) Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. Cell 65:701–713

Moreno S, Nurse P (1990) Substrates for $p34^{cdc2}$: in vivo veritas: Cell 61:549–551

Motokura T, Bloom T, Kim HG, Jüppner H, Ruderman JV, Kronenberg HM, Arnold A (1991) A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature 350:512–515

Mudryj M, Devoto SH, Hiebert SW, Hunter T, Pines J, Nevins JR (1991) Cell cycle regulation of the E2F transcription factor involves an interaction with cyclin A. Cell 65:1243–1253

Nowell PC (1976) The clonal evolution of tumor cell populations. Science 194:23–27

Nurse P (1990) Universal control mechanism regulating onset of M-phase. Nature 344:503–508

Peter M, Nakagawa J, Dorée M, Labbé JC, Nigg EA (1990) In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. Cell 61:591–602

Pfarr CM, Coue M, Grissom PM, Hays TS, Porter ME, McIntosh JR (1990) Cytoplasmic dynein is localized to kinetochores during mitosis. Nature 345:263–265

Pienta KJ, Partin AW, Coffey DS (1989) Cancer as a disease of DNA organization and dynamic cell structure. Cancer Res 49:2525–2532

Pines J, Hunter T (1990) Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. Nature 346:760–763

Reddy AB, Chatterjee A, Rothblum LI, Black A, Busch H (1989) Isolation and characterization of complementary DNA to proliferating cell nucleolar antigen P40. Cancer Res 49:1763–1767

Steuer ER, Wordeman L, Schroer TA, Sheetz MP (1990) Localization of cytoplasmic dynein to mitotic spindles and kinetochores. Nature 345:266–268

Stillman B (1989) Initiation of eukaryotic DNA replication in vitro. Annu Rev Cell Biol 5:197–245

Todorov IT, Philipova RN, Joswig G, Ramaekers FCS (1992) Detection of the 125-kDa nuclear protein mitotin in centrosomes, the poles of the mitotic spindle, and the midbody. Exp Cell Res 199:398–401

Verheijen R, Kuijpers HJH, Driel R van, Beck JLM, Dierendonck JH van, Brakenhoff GJ, Ramaekers FCS (1989) Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J Cell Sci 92:531–540

Wang JC, Caron PR, Kim RA (1990) The role of DNA topoisomerases in recombination and genome stability: a double-edged sword? Cell 62:403–406

Wang J, Chenivesse X, Henglein B, Bréchot C (1990) Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature 343:555–557

Wang TSF (1991) Eukaryotic DNA polymerases. Annu Rev Biochem 60:513–552

Weintraub SJ, Prater CA, Dean DC (1992) Retinoblastoma protein switches the E2F site from positive to negative element. Nature 358:259–261

Wright JA, Smith HS, Watt FM, Hancock MC, Hudson DL, Stark GR (1990) DNA amplification is rare in normal human cells. Proc Natl Acad Sci USA 87:1791–1795