Haematology and Blood Transfusion Vol. 29
Modern Trends in Human Leukemia VI
Edited by Neth, Gallo, Greaves, Janka

Identification of the Human Cellular *myc* Gene Product by Antibody Against the Bacterially Expressed Protein

T. Benter[1], P. Beimling[1], E. Pfaff[2], T. Sander[1], B. Heimann[1], and K. Moelling[1]

Retroviruses code for oncogenes which are related to normal cellular genes. The oncogenes code for products which, according to their properties, can be classified into two groups, one group comprising those gene products which reside in the nucleus, like *myb* and *myc,* and the other, larger group represented by the *src* gene family, which codes for membrane-associated proteins, some of which exhibit protein kinase activities (for review see [4]).

The *myc* gene is the transforming gene of MC29 viruses. Its normal cellular homologous gene may play a role in certain types of tumor such as Burkitt's lymphoma and small cell cancer of the lung (SCCL) [3]; and for review see [6]. To identify the human cellular *myc* (*hu-c-myc*) gene product, the production of antibodies was required. For that purpose a portion of the *hu-c-myc* gene has been cloned into an expression vector for protein expression in bacteria. The expression vector pPLc24 codes for the replicase gene of the bacteriophage MS2 [5] and has been used previously for the expression of a MS2–viral *myc* fusion protein [1] (see Fig. 1, clone *p-myc* 5/30 Sal). The viral *myc* gene was replaced by the ClaI-BclI fragment of the human cellular *myc* gene (clone *p-myc* 6/4, Fig. 1). Expression of the MS2-*hu-c-myc* fusion protein is controlled by a thermolabile repressor. Cells grown overnight at 28 °C are shifted to 42 °C for 2 h which results in expression of the fusion protein. About 10% of the total bacterial protein content is represented by the fusion protein abbreviated as MS2-*myc* in Fig. 2a, which has a molecular weight of about 30 000, 20 000 of which are *myc* specific.

This protein was eluted from gels or purified by differential centrifugation and solubilization in 6 *M* urea. Antibodies were raised in rabbits and the serum applied to immobilized MS2-containing bacterial lysate to remove MS2-specific IgG from the serum and subsequently *hu-c-myc*-specific IgG was recovered from MS2-*hu-c-myc*-containing bacterial protein lysates. Details of a similar IgG isolation procedure have been described [1]. The *hu-c-myc*-specific IgG was used for immunoblotting of HeLa and MC29-Q8-NP cellular lysates which were lysed in RIPA buffer as described [2]. The result is shown in Fig. 2b. The *hu-c-myc*-specific IgG cross-reacts with the $p110^{gag\text{-}myc}$ protein from MC29-Q8 fibroblasts and recognizes a protein of molecular weight 64 000, designated $p64^{hu\text{-}c\text{-}myc}$. A faint larger band of molecular weight 67 000 is also detectable. Figure 3 shows that the *hu-c-myc* gene product in HeLa cells gives rise to nuclear fluorescence. The experiment was performed as described [2]. Whether the *hu-c-myc* gene product is also a DNA-binding protein similar to $p110^{gag\text{-}myc}$ [2], needs to be demonstrated. The *myc*- gene product is expected to be a transcriptional control element. Experiments are in progress to demonstrate this effect.

1 Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63, 1000 Berlin 33, FRG
2 Zentrum für Molekulare Biologie, Universität Heidelberg, Im Neuenheimer Feld 230, 6900 Heidelberg, FRG

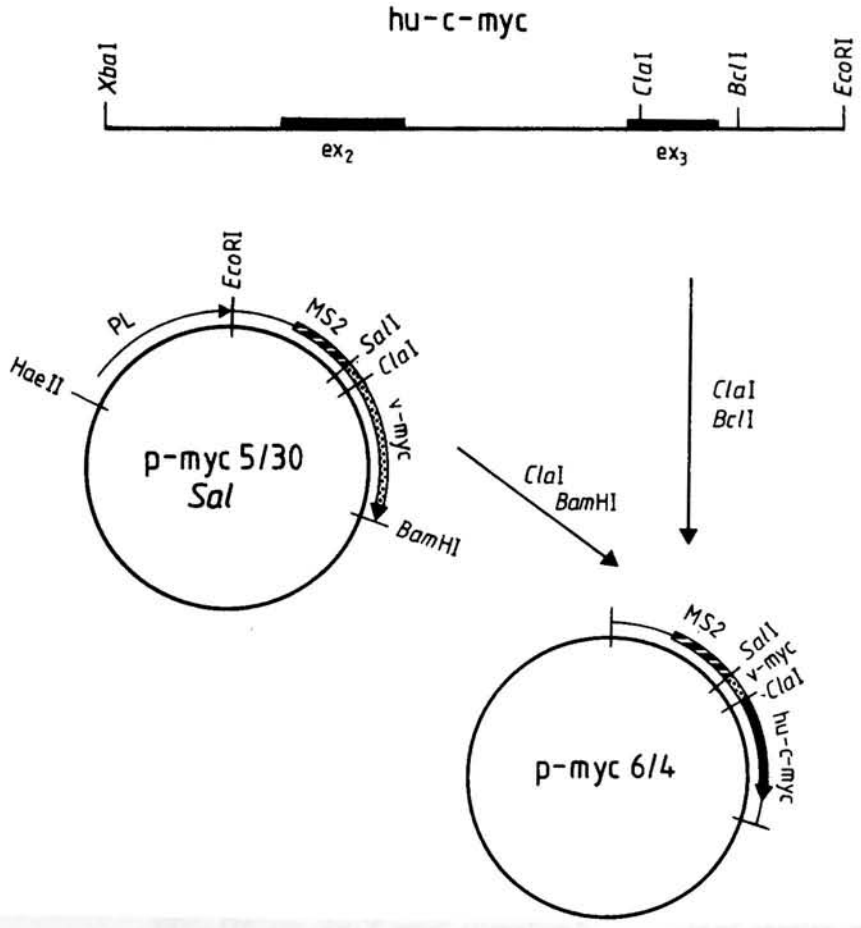

Fig. 1. The *hu-c-myc* clone was constructed from pPLc24 containing a portion of the *v-myc* gene previously designated *p-myc* 5/30 Sal [1]. The *v-myc* gene was replaced by the ClaI/ Bcl fragment of the *hu-c-myc* clone and the clone designated *p-myc* 6/4

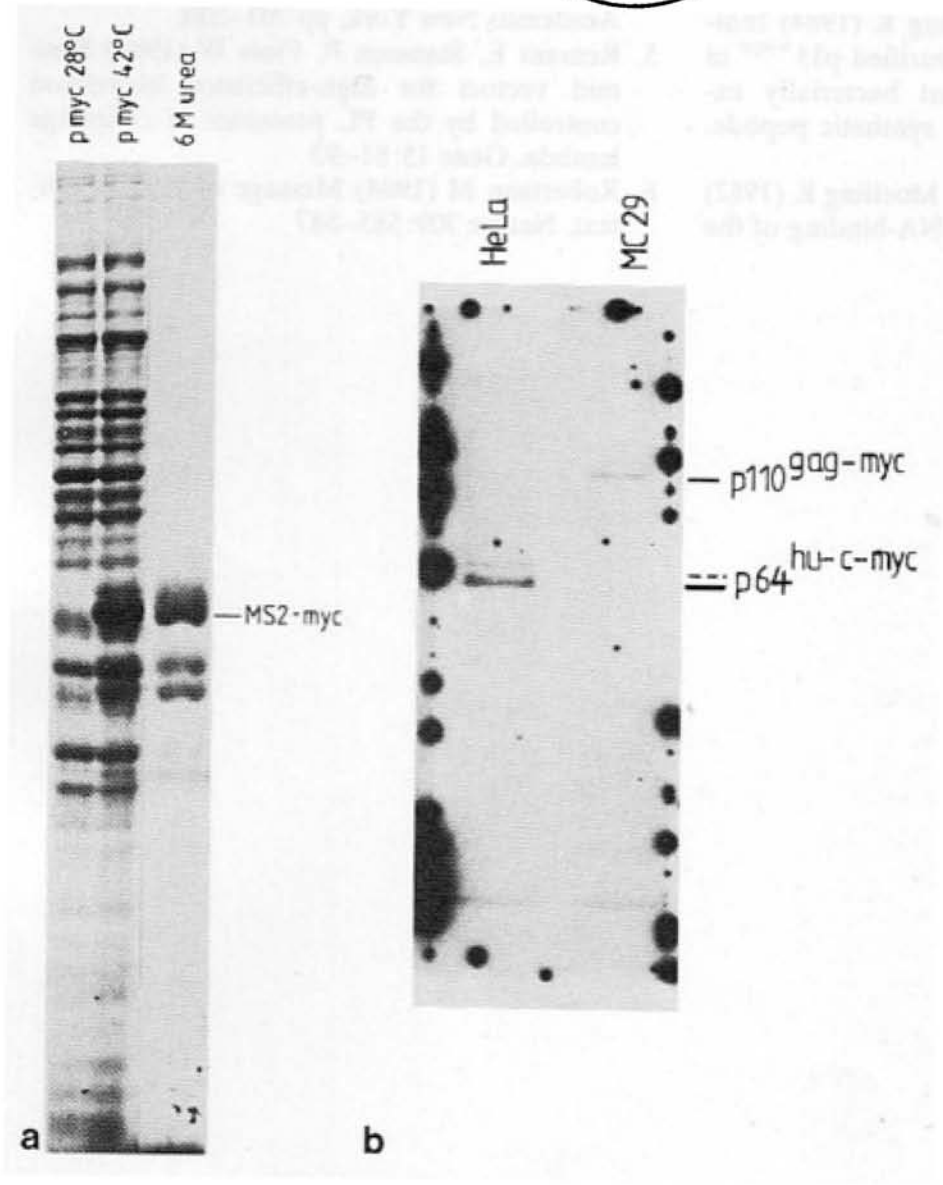

Fig. 2 a, b. **a** The *p-myc* 6/4 clone described in Fig. 1 was expressed in bacteria. At 42 °C, the MS2-*hu-c-myc* protein (abbreviated MS2-*myc*) is expressed. The protein was purified and solubilized in 6 *M* urea; **b** *hu-c-myc*-specific IgG was isolated from antibodies against the MS2-*hu-c-myc* protein and used in an immunoblot with HeLa and MC29-Q8-NP cell lysates

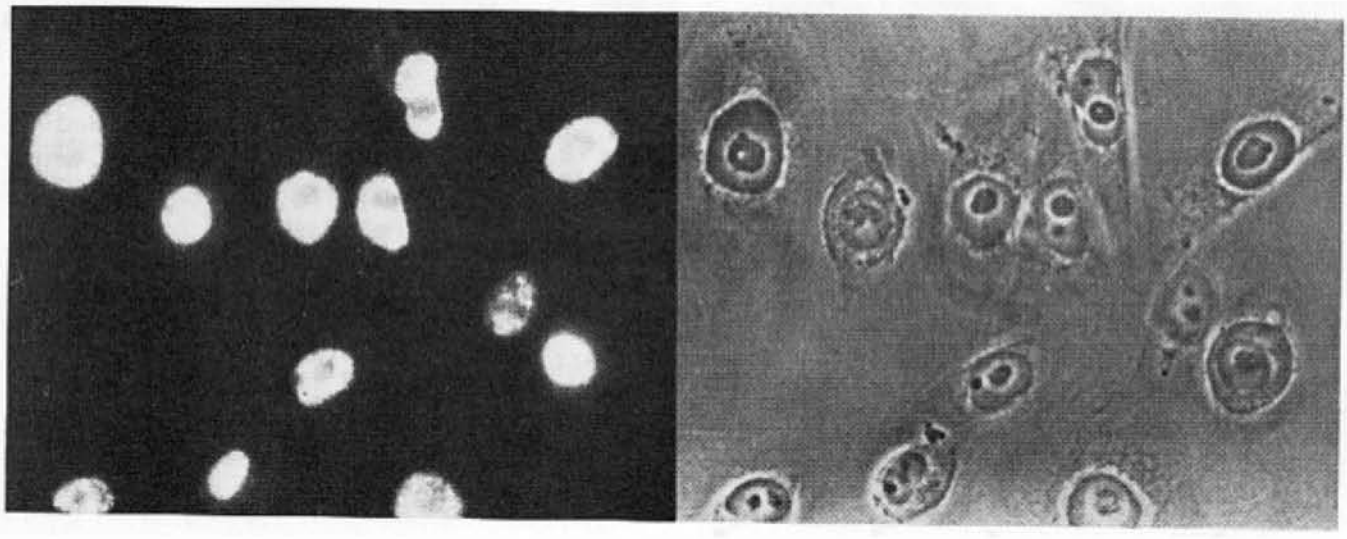

Fig. 3. Indirect immunofluorescence with HeLa cells using *hu-c-myc*-specific antiserum (1 : 40 dilution)

References

1. Bunte T, Donner P, Pfaff E, Reis B, Greiser-Wilke I, Schaller H, Moelling K (1984) Inhibition of DNA-binding of purified $p55^{v\text{-}myc}$ in vitro by antibodies against bacterially expressed *myc* protein and a synthetic peptide. EMBO J 3 (in press)
2. Donner P, Grieser-Wilke I, Moelling K (1982) Nuclear localization and DNA-binding of the transforming gene product of avian myelocytomatosis virus. Nature 296:262–266
3. Little CD, Nau MM, Carney DN, Gazdar AF, Minna JD (1983) Amplification and expression of the *c-myc* oncogene in human lung cancer cell lines. Nature 306:194–196
4. Moelling K (1985) Fusion proteins in retroviral transformation. In: Weinhouse S, Klein G (eds) Advances in cancer research, vol 43. Academic, New York, pp 205–239
5. Remaut E, Stanssen P, Fiers W (1981) Plasmid vectors for high-efficiency expression controlled by the PL promoter of coliphage lambda. Gene 15:81–93
6. Robertson M (1984) Message of myc in context. Nature 309:585–587