fibroblasts (MEF) counterpart (about 10 population doublings). It seems that the frequency of immortalization of cells is inversely correlated to their *in vitro* lifespan, because human (primate) and chicken cells immortalize very rarely in comparison with mouse (rodent) cells (Hayflick, 1965; Beug and Graf, 1977).

Although the molecular mechanisms of senescence are not fully understood, some signalling pathways have been outlined. At least two ways are branched from the gene *Ink4a/Arf* coding for two different proteins $p16^{Ink4a}$ and $p19^{Arf}$. The expression of each protein, of totally different function, is controlled by a separate promoter (Quelle et al., 1995; Sharpless and DePinho, 1999). $p16^{Ink4a}$ inhibits cyclin-dependent kinases (cdks) 4/6, which in conjunction with cyclin D1 initiate, in the early G1 phase, phosphorylation of the retinoblastoma (Rb) protein, critical for progression through the cell cycle (Sherr, 1994; Morgan, 1995). $p19^{Arf}$ interacts with MDM2, abrogating its induction of p53 degradation mediated by the ubiquitin-proteasome pathway, thus functioning as an inhibitor of inhibitor. The accumulated p53 then transcriptionally activates the $p21^{Waf1/Cip1}$ inhibitor of cdk-cyclin complexes (Harper et al., 1993; Xiong et al., 1993; Quelle et al., 1995). Telomere shortening is another mechanism of senescence important in human cells (Sedivy, 1998), but not much in mouse or hamster cells with long telomeres and constitutive expression of telomerase, which can prevent telomere shortening (Blasco et al., 1997; Carman et al., 1998; Russo et al., 1998).

Kim et al. (2001a) have demonstrated that inactivation of the *p53* gene occurs at the post-transcriptional level by rapid destabilization of its mRNA in the nucleus of spontaneously immortalized MEF cells. Functional p53 activity was shown to increase in the replicative senescent HDF and CEF, but not in senescent MEF cells. On the other hand, there was an elevation of p16(INK4a) expression in replicative senescent MEF cells. In contrast to the HDF and CEF cells, MEF cells were shown to express telomerase mRNA and maintain the telomerase activity throughout their *in vitro* life-span. Simultaneously, HDF and CEF were proved to have a more extended *in vitro* proliferative potential than MEF (Kim et al., 2002). Results of different groups have shown that young and senescent human diploid fibroblasts (HDF) respond differently to DNA damage induced by a variety of genotoxic stresses. Young fibroblasts undergo apoptosis either in p53-dependent or independent manner. Senescent HDF, instead of undergoing p53-dependent apoptosis, die by an alternative pathway, necrosis (Seluanov et al., 2001). The results suggest that stabilization of p53 in response to DNA damage is impaired in senescent fibroblasts, resulting in induction of necrosis.

Recently, several non-transformed immortalized chicken embryo fibroblast (CEF) cell lines have been established in continuous cell culture. Cells were taken from a chicken line free of endogenous retroviral loci (Himly et al., 1998; Schaefer-Klein et al., 1998). All immortal CEF cells tested showed common genetic alterations in the expression patterns of p53 and E2F-1 mRNA and protein, which were down- and upregulated, respectively (Kim et al., 2001b, 2001c). Destabilization of p53 mRNA was observed in the nuclei of immortal, but not primary, CEF cells. The half-life of p53 mRNA in primary cells was found to be relatively long, 23 h compared to only 3 h in immortal cells. Taken together, the results suggest that the downregulation of p53 mRNA in immortal CEF cells occurs through a post-transcriptional destabilizing mechanism. In addition, expression of most of the cyclin genes was upregulated in immortal CEF cells, which may be associated with the rapid cell division rates and serum-independent growth patterns seen in immortal CEF cells. The telomeric lengths and chromosome integrity were maintained in all immortal CEF cell lines without detectable telomerase activity (Kim et al., 2001c). While loss of p53 function due to mutations of critical residues is frequent in cancer and immortal cells (Levine et al., 1991; Cho et al., 1994), the genetic changes leading to alterations of p53 through transcriptional and post-transcriptional regulation seem to be quite unique to immortal CEF cells.

Despite the immortalization status, the DF-1 line, one of the immortalized CEF lines, responds in a similar way as normal CEF to infection with avian leukosis-sarcoma viruses of different subgroups and to the expression of their v-*onc* genes. Immortal DF-1 cells also were, like human immortal cells, efficiently transformed by v-*ras* (Himly et al., 1998). Expression of the growth suppressor gene *p21waf1* blocks the replication of CEF transformed by several oncogenes (Givol et al., 1995, 1998) and also causes growth arrest of immortal DF-1 cells (Schaefer-Klein et al., 1998). One unexpected difference between the DF-1 and normal CEF concerns the cytopathogenic effect of envelope subgroup C ALVs, which are not normally cytopathic to CEF. In contrast, the DF-1 cells are severely affected by subgroup C viruses, which induced extensive cell death by apoptosis (Himly et al., 1998). Envelope subgroup B and D viruses induced apoptosis in both CEF and DF-1 cells. The apoptosis-inducing activity of these two subgroups of viruses is mediated by a cell surface receptor related to the receptor for tumour necrosis factor (Brojatsch et al., 1996). The cell-killing activities of envelope subgroup C viruses in DF-1 cells remain to be explained, because the molecular structure of the subgroup C receptor is not yet known.

Genetic changes leading to immortalization of the chicken cells transformed by (*ts*) v-*onc* genes have been studied by Ulrich et al. (1992). Loss of *p53* mRNA or expression of a mutated *p53* gene invariably occured in the early phase of immortalization. In contrast, expression of the *Rb* gene was unchanged at all stages of immortalization. Immortal clones of mortal chicken fibroblasts and erythroblasts transformed by tempera-

ture-sensitive v-Src and v-ErbB oncoproteins retain the conditional transformed phenotype despite the lack of p53. The loss of p53 function did not enhance or alter the transformed phenotype of cells, either. These results demonstrate the independence of processes of immortalization and transformation in this model.

Several transplantable tumours and tumour cell lines harbouring the LTR, v-*src*,LTR provirus were established in our laboratory (Svoboda et al., 1992, 1996; Plachý et al., 1994; Plachý, 2000). For tumour induction in chickens, we employed the molecularly cloned LTR,v-*src*,LTR proviral DNA derived from the H-19 hamster tumour line (Bodor and Svoboda, 1989), the sequence of which exactly matches the reverse transcript of v-*src* mRNA. Long terminal repeats (LTRs) comprise strong promoter-enhancer sequences and ensure efficient expression of the v-*src* gene. The integrity of the LTR,v-*src*,LTR provirus and the high level of v-*src* expression were found in all transplantable tumours and derived immortal tumour cell lines. Interestingly, quite a high immortalization efficiency was observed in cells from *ex vivo* tumours growing progressively in chickens of the inbred line CC.R1 (B^{4r1}/B^{4r1}). This chicken line is the most susceptible to v-*src*-induced tumorigenesis among the Prague MHC(B) congenic lines due to an inappropriate immune response to v-Src antigens (Plachý et al., 1994). About 20% of CC.R1 progressing tumours are also repeatedly transplantable in syngeneic hosts and all these tumors gave rise to an *in vitro* permanently growing tumour cell line. However, there still remains the majority of tumors also growing progressively in a primary host, which are not transplantable, and the derived cells show no extended life-span *in vitro*. Thus, v-*src*-transformed cells can be relatively easily immortalized, but cell immortalization is not necessary for primary tumour outgrowth and further progression in the tumour host.

Another example of independence between processes of transformation, tumour progression and immortalization is provided by our experiments with a new variant of the *src* gene, the PR2257 v-*src* (Svoboda et al., 1996, and unpublished results; for the structure of PR2257 v-*src* see Geryk et al., 1989). In this case, we have never succeeded with prolonged *in vitro* passaging of PR2257 v-*src* DNA-induced tumours, despite their high oncogenicity in the CC.R1 chickens causing a rapid progression. During early passages, the cells synthesized large amounts of mucopolysaccharide, which covered the culture surface with a macroscopically visible layer and developed the characteristic vacuolated giant cell appearance of ageing cells (Fig. 1).

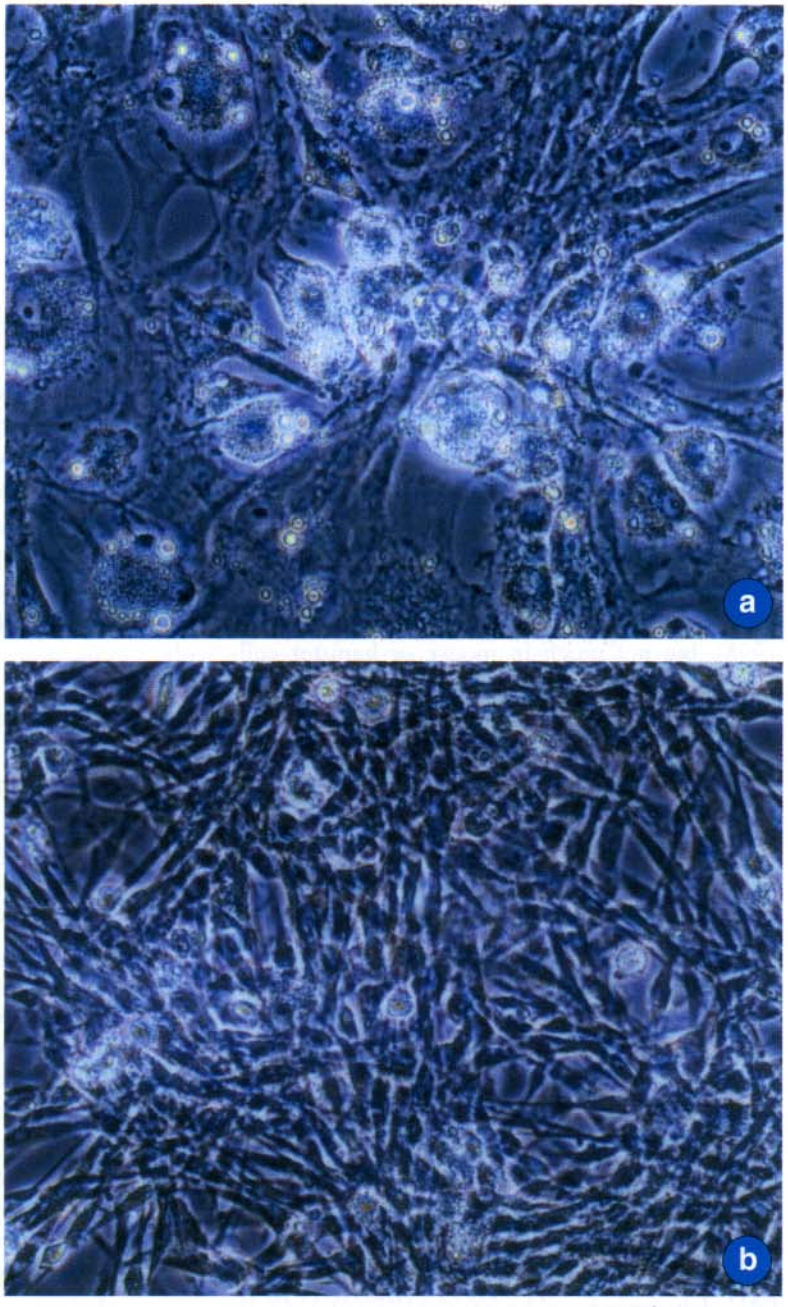

Fig. 1. Typical morphology of tumour cells derived from the PR2257*src*- **(a)** or v-*src*- **(b)** induced tumours.
a – 5th passage of tumour cells derived from the PR2257*src* DNA-induced tumour in a chick of the CC.R1 inbred line. Characteristic vacuolated giant cell appearance of ageing culture.
b – Confluent culture of a long-passaged cell line (PR6286M) derived from lung metastasis of the LTR,v-*src*,LTR DNA-induced tumour in a chick of the CC.R1 inbred line. Picture illustrates the relatively uniform appearance established at confluency and multilayer growth. Before culture becomes confluent, cells appear pleomorphic, with round and fibroblast-like appearances predominating.