Programmed cell death in the reproductive system

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The reproductive system presents some of the best examples of programmed cell death, which is to be expected considering the dramatic cycles of tissue growth and regression in females. Hormones from the pituitary gland, gonads and uterus are responsible for coordinating cycles in which the preservation of cell survival and inhibition of apoptosis are important. In the ovary, atresia regulates the size of the follicle cohort for ovulation and is an archetype of apoptosis as induced by hormone withdrawal. The fate of an antral follicle — growth or atresia — is determined by circulating levels of gonadotrophins, and follicle-stimulating hormone (FSH) in particular. At the end of a menstrual cycle or pregnancy or lactation, hormone withdrawal triggers cell death and tissue remodelling and initiates a fresh cycle. In the endometrium, breast and prostate gland, steroid hormones are the principal survival factors and castration triggers regression of responsive tissues, which is sometimes decisive in the fight against disease. But while the primary trigger of cell death varies between tissues, underlying cellular mechanisms are more conservative and cell death/survival genes, such as bcl-2, bax and others that are expressed in other tissues, play important roles in the reproductive system too.

Apoptosis or programmed cell death is more obvious and has been more intensively investigated in the reproductive organs than in almost any other system. Indeed, dying cells were described in the Graafian follicles of rabbit ovaries as long ago as 1885, although the phenomenon was then called ‘chromatolysis’ rather than apoptosis. One explanation for cell death in the reproductive system is that it is required because of the cyclical growth and re-modelling of tissues occurring throughout adult life — at least until menopause in females. Another reason is that far more germ cells are produced than are needed. The gonads are not the only organs generating excess cells, though they are unusual insofar as the wastage continues on a large scale in adult life. In the endometrium, epithelial and stromal tissues grow and differentiate until the menstrual cycle is terminated by programmed regression of the functional layer — unless conception occurs first. Parallel, though less obvious, changes occur in breast and other tissues, the whole process being coordinated by pituitary and ovarian hormones. Few systems, if any, provide such clear demonstrations of the pivotal role of hormones in determining cell fate.
And sex steroids and progesterone emerge as major survival factors in the reproductive system, in contrast to the glucocorticoids which trigger apoptosis in the immune system.

This canon of reproductive endocrinology holds true of males as well as females. While the fate of spermatozoa is programmed in the sense that they cannot repair cellular damage, immature germ cells are eliminated by processes resembling apoptosis. What is more, prostatic regression after orchidectomy or treatment with androgen antagonists is a programmed event and illustrates how hormonal manipulation can bring therapeutic benefit.

In an article of this length, it is not possible to describe every aspect of the system and, to make our task more manageable, we have concentrated on the primary organs of reproduction—the gonads and genital tract—and have ignored the pituitary gland, the hypothalamus, adipose tissue and skin despite the fact that they are all responsive in some degree to sex hormones. Our account begins in the embryo because the size of the founding populations of germ cells lays foundations for fertility and sex hormone sufficiency.

**Development of the reproductive organs**

Primordial germ cells are visible in the yolk sac at 4 weeks of gestation and shortly afterwards migrate to the gonadal ridge. Their numbers are maintained by cytokines rather than by pituitary gonadotrophins and sex steroids which play their roles at a later stage. The gonads are sterile in animals with either inactivating mutations at the *steel* locus, which encodes Kit-ligand (stem cell factor) in neighbouring somatic cells, or at the *W* locus, which encodes its cognate receptor, c-Kit, in the germ cells. These factors continue to play a role after birth by promoting oocyte growth and spermatogonial survival.

Germ cells continue to proliferate after settling in the gonads, which do not become sexually dimorphic until 6 weeks of gestational age. Peak numbers of about 7 million cells are found in the ovary at mid-gestation, and mitotic oogonia coexist with oocytes at various stages of meiotic prophase. In the second half of pregnancy, the numbers decline precipitously and oogonia are virtually extinct at birth with oocytes falling to 1–2 million in toto. Many cells die at the pachytene stage though moribund germ cells are observed at almost every other stage, including mitosis. Some germ cells die in the ovarian parenchyma after failing to become primordial follicles, whereas others migrate through the ovarian epithelium into the peritoneal cavity. Apoptosis is thought to be at least partly responsible, which would be consistent with germ cell
deficiency in transgenic mice lacking the apoptosis-suppressing gene, \textit{bcl-2}. It remains unproven whether cells are eliminated selectively on the basis of ‘quality’, though a high frequency of chromosomal errors in fetal germ cells and a germ cell deficiency in aneuploid fetuses (notably 45,XO) point to this conclusion. Not all oocytes that perish are necessarily abnormal though, and wastage may be a mechanism for regulating the numbers of normal cells, by analogy with the elimination of surplus motor neurons in the developing spinal cord. If the cells dying are healthy and the process was preventable, a much larger follicle store could be established and menopause would presumably be rescheduled to later in life.

During the sexually indifferent stage of fetal life, a pair of Wolffian ducts and Müllerian tubes coexist but only one set survives depending on prevailing levels of androgens and Müllerian-inhibiting substance (MIS), respectively\textsuperscript{7}. Thus, in males the ducts survive to form part of the male tract while the tubes disappear in response to MIS. In females, the opposite set survives to form the uterus, cervix and upper vagina. These changes, which have all the appearance of programmed cell death, are ripe for further investigation.

\section*{The ovary}

\textit{Follicular dynamics}

After birth, most oocytes do not reach maturity but are discarded with their granulosa and thecal cells in atretic follicles. By puberty a child’s ovaries have already lost three-quarters of the follicle store that was present at birth. Between puberty and menopause there is the potential for up to 500 spontaneous ovulations: the great majority of the 250,000 follicles remaining is eliminated by atresia. The rate of depletion of the store of primordial follicles is constant until 37 years of age when it accelerates and, thereby, hastens the onset of menopause (Fig. 1)\textsuperscript{8,9}.

About 1000 follicles begin growing during a typical menstrual cycle in young women but only one or two are selected for ovulation. While it is still not clear what triggers follicle growth or whether selection is at random, there is no doubt that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are required for maturation and ovulation. There are receptors on the granulosa and theca cells and the hormones have several roles, including stimulation of follicle growth, steroid production, ovulation and maintenance of the corpus luteum. The ovulation rate is rather precisely controlled by FSH and hypophysectomy.
Follicular atresia

At the beginning of the menstrual cycle, serum FSH concentrations are slightly elevated and one (occasionally two) follicle becomes dominant, producing 95% of the circulating oestradiol and reaching maturity at mid-cycle. FSH secretion falls in response to higher serum oestrogen and inhibin, and other members of the follicle cohort become atretic as a result of gonadotrophin deprivation, possibly reinforced by inhibitory factor(s) from the dominant follicle. Follicles destined for atresia can be rescued by boosting gonadotrophin levels with clomiphene citrate or exogenous hormones to yield as many as 10 or more follicles for ovulation or egg collection in *in vitro* fertilisation treatment. Atresia, therefore, is the default pathway for follicle development rather than a reflection of the quality of the oocyte inside (Fig. 2).

Small follicles rarely undergo atresia spontaneously. Most atretic follicles are in the 1–5 mm diameter range when they are acutely dependent on FSH for survival and their numbers are being regulated for ovulation (Fig. 3). These antral follicles and the larger Graafian stages are absent in the ovaries of hypogonadotrophic patients and after treatment with GnRH analogues. In polycystic ovarian syndrome, follicles usually halt at or before the 10 mm size and fail to become oestrogenic or to ovulate. While usually regarded as a disorder of
Follicular development

Apoptosis

Follicular development, it could be regarded as a syndrome of repressed atresia because cystic follicles accumulate in the ovary despite relatively low FSH levels.

Atresia is so central to the proper function of the ovary and so familiar that it has often been used as a model for investigating the role of genes involved in apoptosis and for testing methods for revealing DNA...
fragmentation. Pyknosis of granulosa cells occurs at early stages of atresia and the fate of the follicle is still reversible by gonadotrophin treatment or even by explanting in vitro. But, as cell death becomes more widespread, mitosis ceases, gap junction communication is lost, thecal cells hypertrophy and the basement membrane breaks down. P450 aromatase activity declines and granulosa cells produce progesterone instead of oestradiol. The oocyte appears to be healthy until late stages of atresia when it may spontaneously resume meiosis, expel a polar body and reach metaphase II. Although resembling nuclear maturation in periovulatory follicles, meiosis is evidently triggered by loss of inhibitory influences as the cumulus cells detach—hormonal stimulation is evidently not required.

Follicular atresia involves a stereotyped succession of biochemical and morphological events, including cleavage of DNA by Ca+2/Mg+2-dependent endonucleases into multiples of 185-200 bp length. This classical 'DNA ladder' is revealed by agarose gel electrophoresis and corresponds to apoptotic bodies observed microscopically. Apoptosis can be completed within minutes in single cells, but the breakdown and disappearance of a whole follicle takes several days, according to size.

Hormonal regulation of atresia

FSH is the most important follicle survival-promoting hormone in vivo and in culture and there is a notable decrease in FSH receptors on granulosa cells during atresia. Nevertheless, the process of atresia is far from simple and a complex balance of survival and atretogenic factors determine follicle fate (Table 1). For practical reasons, most experiments have been based on immature or hypophysectomized rats treated with diethylstilboestrol, but the relevance of this model for other species should not be assumed automatically. From these studies, it is emerging that paracrine factors, such as insulin-like growth factor-I (IGF-I), interleukin-1β, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) sway follicle survival, and this is likely to be true for most if not all species. Some paracrine factors mediate the effects of peripheral hormones as well as moderating or enhancing the actions of others. For instance, IGF-I protects follicles from undergoing atresia, though this action is blocked by the binding protein, IGFBP-3. Since IGFBP-3 partially reverses gonadotrophic action, FSH is probably mediated by the IGF-1 receptor tyrosine kinase system. EGF and bFGF also have protective effects on follicles, and these can be blocked by the tyrosine kinase inhibitor, genistein; interleukin-6 and androgens, on the other hand, induce atresia by direct effects. Gradually, a balance
Table 1  A catalogue of hormones, cytokines and genes that favour follicular and oocyte survival compared with atretogenic factors

<table>
<thead>
<tr>
<th>Survival factors</th>
<th>Atretogenic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadotrophins (especially FSH)</td>
<td>IGF binding proteins</td>
</tr>
<tr>
<td>IGF-1, GH</td>
<td>TGFβ</td>
</tr>
<tr>
<td>EGF/TGFα</td>
<td></td>
</tr>
<tr>
<td>VIP, XGP, HGF, bFGF</td>
<td>IL-6</td>
</tr>
<tr>
<td>II-1β, nitric oxide</td>
<td>Androgens</td>
</tr>
<tr>
<td>Oestrogens</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Free radical scavengers</td>
<td>GnRH</td>
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<td></td>
<td>box</td>
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<tr>
<td></td>
<td>fas antigens</td>
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<tr>
<td>bcl-2, bcl-xinv</td>
<td>p53</td>
</tr>
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<td></td>
<td>ICE-related proteases</td>
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sheet of survival-promoting and atretogenic factors is being drawn up and the challenge is to discover how they interact physiologically and apply this knowledge to achieve finer control of stimulated ovarian cycles.

There is evidence that a balance of steroid effects determines follicular fate—the atretogenic actions of androgens contrasting with the protective effect of oestrogens in rats. This neat theory is called into question in human ovaries where there is a paucity of oestrogen receptors in granulosa cells and follicles stimulated using pure, recombinant FSH grow despite the production of much less oestrogen than normal. Additionally, androgens can stimulate follicle development in some circumstances and apoptosis in others and the ability of GnRH agonists to induce atresia directly in rats is a further illustration of the complexity of atresia. Just as hormones have pro- and anti-atretic effects, certain genes induce apoptosis in granulosa cells whereas others are protective.

Gene action

The molecular basis of follicular development is being explored by investigating genes that are known to affect cell fate in other systems and physiological significance is tested most revealingly using targeted mutagenesis and transgenic animals. bcl-2 is a proto-oncogene encoding an intracellular protein that prevents apoptosis induced by a number of agents and is important in the reproductive system too. A deficiency of bcl-2 leads to formation of a smaller follicle store whereas excess expression in the ovary causes a higher ovulation rate, indicating that atresia is down-regulated. Ovaries from null mutants for the bax gene,
which can induce apoptosis by suppressing bcl-2, contain abnormal follicles with excess granulosa cells. The bax protein is apparently involved in granulosa cell apoptosis in follicles undergoing atresia, though its absence does not prevent the induction of atresia, indicating that follicular atresia and granulosa cell apoptosis may not be strictly equivalent. When trophic support is withdrawn, an even more marked decline occurs in the mRNA levels of the related survival-inducing gene bcl-x\textsubscript{long}—in contrast to the rising levels of bax. 

Fas/APO/CD95 is another potentially important factor in atresia. It is a member of the gene family for receptors of tumor necrosis factor and nerve growth factor and is characterized by a conserved ‘death domain’ motif which is vital for apoptosis in lymphocytes and other cells. It now appears to include animal and human ovaries in which Fas antigen is expressed in the granulosa cells. Not every gene implicated in cell death elsewhere necessarily has a role in the ovary. Interleukin-1\beta-converting enzyme (ICE), the mammalian homologue of the apoptosis-inducing ced-3, apparently plays no role although the ICE-related Ich-1\textsubscript{long} gene and CPP32 are probably more significant. We must also bear in mind that genes may exist that are involved only in follicle survival, though none have yet come to light.

Cytotoxic damage

Ovarian follicles are remarkably vulnerable to agents that cause DNA damage such as ionizing radiation and chemotherapeutic drugs. Primordial follicles in mouse ovaries are among the most radiosensitive of all mammalian cells and have an LD\textsubscript{50} of only \( \sim 0.5 \) Gy. Although human follicles are only a tenth as sensitive, their sensitivity falls into the therapeutic range and young patients are frequently rendered sterile by cancer treatment. The impact of radiotherapy and chemotherapy varies with age, and women over 30 years old are more likely to be sterilized than younger ones or children receiving the same dose because they have fewer follicles remaining (Fig. 1). Somewhat paradoxically, growing follicles are less radiosensitive than the quiescent primordial stage. The response of primordial follicles to cytotoxic injury is often assumed to be apoptotic though it has rarely been observed. Whether the tumour suppressor p53 is involved in follicle demise is not known though p53 does depress the activity of bcl-2 and increase transcription of bax in the rat ovary. What is more, under experimental conditions p53 is altered by gonadotrophin treatment and overexpression can induce apoptosis in cultured granulosa cells.
The possibility of suppressing follicle wastage and protecting fertility in cancer patients is currently being discussed because more young women and men are now surviving cancer after treatment with aggressive high-dose chemotherapy and bone marrow transplantation. Administration of GnRH agonists has sometimes been found to moderate cytotoxic damage in animal ovaries, though the mechanism is not understood and is possibly independent of the gonadotrophin levels. The clinical benefits of such treatment remain unproven. Moreover, such a strategy may be ill-advised even if it works if it prevents apoptosis in germ cells harbouring damaged DNA.

**Luteolysis**

The corpus luteum is an endocrine organ formed after ovulation and with a lifespan which is fixed unless stimulated by chorionic gonadotrophin (hCG) from an implanted conceptus. The identity of the factor(s) responsible for luteolysis is poorly understood in primates compared with the evidence of involvement of prostaglandins from the uterus in some domestic species of animals. Cytological changes resembling apoptosis occur in luteal cells undergoing structural luteolysis according to some recent studies, though the character of these changes is less clear-cut than in the follicle and necrosis is also found. The proto-oncogene c-myc, which has been implicated elsewhere in both mitosis and apoptosis, is of questionable importance in monkeys since it is present in the corpus luteum throughout the luteal phase and does not disappear during regression, and bcl-2 presents a similar picture in human cells. Somewhat more intriguingly, c-myc is not expressed in human luteal cells until luteolysis. On the basis of such limited information it is difficult to draw firm conclusions, and the biology of human luteolysis is still far from understood.

**The testis**

The testes in normal men produce about $10^8$ spermatozoa daily. This output depends on proliferative activity in the basal compartment of the seminiferous epithelium where the spermatogonial cells are found and differentiation towards the lumen where meiosis and spermiogenesis occur. Programmed cell death is less obvious than in the ovary, at least in healthy adult organs, though careful examination reveals some apoptotic
bodies and low molecular weight DNA. The significance of regulating the cell population by apoptosis is more apparent when sperm production is halted.

Secretion of both pituitary gonadotrophins and testosterone is at a low level before puberty and in seasonally breeding animals whose testes are regressed. Full spermatogenesis awaits a rise in hormone levels though the germ cell population is not completely quiescent and development terminates without passage through stages of meiosis. Likewise, hypophysectomy or the administration of GnRH analogues thwarts spermatogenesis and causes the seminiferous epithelium to atrophy and germ cells to degenerate. Some debate exists about the exact definition, but it is thought that the process of cell death involves apoptosis under these circumstances and after chemical injury (Fig. 4). Some stages of spermatogenesis are affected earlier than others though all are vulnerable and spermatogonia die as they enter mitosis. Hypogonadism caused by hypopituitarism is reversed by injecting exogenous hormones or suspending GnRH administration. These findings imply that germ cells are as dependent on gonadotrophins for survival as they are for stimulating proliferation and differentiation. Therefore, regulation of cell survival is now regarded as a key factor in controlling the rate of production of spermatozoa, which parallels the actions of gonadotrophins in preventing follicular atresia.

![Fig. 4 Gonadotrophin suppression of hypophysectomy-induced testicular apoptotic DNA fragmentation.](image)

**Fig. 4** Gonadotrophin suppression of hypophysectomy-induced testicular apoptotic DNA fragmentation. Immediately after hypophysectomy of 21-day-old rats, 20 IU FSH-CTP or 50 IU hCG were injected subcutaneously at 24 h intervals, and the animals were killed 2 days after the first injection. Testicular DNA was labelled on 3' ends with \( \alpha^{32}P \)-dideoxy-ATP and analyzed by autoradiography (A) and \( \beta \)-counting of low-molecular weight DNA fractions (B). INT, intact; HPX, hypophysectomized; C, control, FSH, FSH-CTP. *P < 0.001 vs control. Reprinted with permission from Billig et al. [5].
Despite the importance of this process, there have been fewer studies of the molecular basis of cell survival in the testis than in the ovary. In a recent study of transgenic mice targeted expression of bcl-2 produced seminiferous tubules accumulating enormous numbers of spermatogonia, suggesting that this gene is normally involved in suppressing cell death, as in other organs. As elsewhere, the actions of bcl-2 are opposed by bax because mice made bax-deficient are sterile and have defective premeiotic germ cells. Evidently, it is unsafe to extrapolate gene action between sexes because the female bax transgenic mice had relatively normal follicle development.

A number of other factors besides hormones can trigger regression of the epithelium and render the testis sterile. Destruction of Leydig cells in rat testes using glycol ethers causes germ cell death but, since the stages affected first are different, a decline in testosterone may not be solely responsible. Scrotal testes are maintained several degrees cooler than body core temperature and local heating suppresses spermatogenesis and fertility. The first cells to die when testes are made cryptorchid are pachytene spermatocytes and early spermatids, followed by other cell types. The appearance of heterochromatic bodies and DNA fragmentation indicates that an apoptotic process is involved.

As expected in proliferating cell types, spermatogenesis is highly sensitive to agents that cross-link and break DNA strands, such as alkylating agents and radiation. Depending on the dose and treatment protocol, there is often a lull in sperm production in cancer patients receiving chemotherapy or radiotherapy, if not complete and irreversible aspermatogenesis. Differentiating spermatogonia are killed at doses of <3 Gy, though stem cells are more resistant. Even so, restoration of spermatogenesis is often incomplete and tubules sometimes remain permanently azoospermic despite the presence of residual spermatogonia.

Finally, we venture to suggest an explanation for the remarkable success story of chemotherapy for testicular tumours, which can cure even large, disseminated masses. Perhaps it is significant that teratocarcinomas and seminomas express wild-type p53 protein highly and without producing mutations affecting gene function. The efficacy of combination chemotherapy could be owing to the ability of agents like etoposide to activate latent p53 and so induce apoptosis.

**The female reproductive tract**

Cyclical growth and secretion of the endometrium during the menstrual cycle are regulated by ovarian steroids in combination with cytokines,
whereas its regression is programmed by local production of prostaglandins. The survival promoting action of progesterone is antagonized by mifepristone (RU-486), which increases epithelial cell death and stromal compaction in the monkey uterus. The effects of RU-486 may not be only indirect because they persist in the absence of progesterone\textsuperscript{53}.

The protein bcl-2 is expressed in the glandular epithelium and spiral arteries during the follicular phase when serum oestrogen levels are rising. Its disappearance during the luteal phase shortly before menses implies that it could be hormonally controlled and have a role in preventing premature cell death and endometrial shedding\textsuperscript{54}. What is more, persistence of bcl-2 in stromal, lymphoid and epithelial cells of the zona basalis might account for the survival of these privileged cells, which repair the endometrium after menses\textsuperscript{55}. In the vagina, cyclical regression is accompanied, as might be anticipated, by lower bcl-2 and higher levels of the apoptosis-inducing Fas ligand\textsuperscript{56}. So far, the physiological significance of apoptosis has been more-or-less clear but it is difficult to say whether the occurrence in blastomeres of preimplantation human embryos conceived \textit{in vitro} is a sporadic effect or a sign of poor health. Early embryos can compensate for the loss of one or two cells at the morula stage. The triggering of programmed cell death by the blastocyst as it buries itself in the uterine epithelial cells is more understandable. These cells become detached from the basement membrane to give access to the underlying decidual cells—some of which also die to accommodate expansion of the conceptus\textsuperscript{57}. Thus, apoptosis occurs in the presence of high levels of progesterone which promotes decidual cell survival until levels fall. Dying cells are rapidly phagocytosed by neighbouring cells without an accompanying inflammatory reaction.

The mammary gland

The mammary and prostate glands bear close comparison for, although hormonal requirements for function differ, they both depend on sex steroids for growth. Breast tissue comes under the strong influence of ovarian steroids for the first time during sexual maturation, although the lobulo-alveolar glands are not fully developed and secretion does not occur until the end of pregnancy. After lactation has ceased, the gland undergoes dramatic involution involving suppression of the $\beta$-casein gene and regression of the glandular epithelium, endothelial and
Apoptosis

myoepithelial cells by an apoptotic process that is almost entirely absent in the functioning gland. In laboratory animals, regression is so complete that the gland returns to a virgin state. Such radical remodelling of tissue requires breakdown of extracellular matrix and loss of cell adhesion by the actions of metalloproteinases, including stromelysins and gelatinase and urokinase-type plasminogen activator. Expression of interleukin-1β converting enzyme (ICE) seems to be important for cell death to occur and is suppressed by components of the basement membrane. Evidently, the interplay between epithelial and mesenchymal cells is as complex in the process of involution as it is in maturation.

Ionizing radiation, chemotherapeutic agents, heat treatment and hormone ablation owe much of their therapeutic benefit in controlling tumour growth to apoptosis. The extent to which breast cancer responds to oestrogen withdrawal by cytostasis as opposed to apoptosis is not clear, though cell death is a feature of human mammary xenograft models. After removing an oestrogen implant from nude mice, more nucleosomal oligomers appear and transforming growth factor-β is expressed, as is the TRPM-2 gene which, though first described in regressing prostatic tissue, is turning out to have wider significance in hormone-dependent tissues. The survival-enhancing gene bcl-2 is expressed in normal breast tissue as well as in cancer cell lines and may be oestrogen-responsive since patients with higher levels derive more benefit from endocrine therapy. The Fas antigen and p53 protein evidently have important roles in apoptosis. Mutations are common in p53 in breast tumours and may confer tumour resistance to DNA-damaging agents.

The prostate gland

The prostate gland grows to mature size under the influence of androgens, specifically 5α-dihydrotestosterone, which stimulate mitosis and inhibit programmed cell death. Prostate tumours are almost unknown in castrates. They are initially androgen-sensitive and a reduction in hormone levels triggers glandular involution. Unfortunately, these palliative effects are temporary and the disease generally becomes recrudescent by evolving to a state of androgen-independence which is resistant to treatment and eventually proves terminal.

Apoptosis in the normal gland involves two stages (Fig. 6). Tall, columnar cells in the functioning gland shrink and secretion eventually ceases. There are marked increases as well as some decreases in gene
transcription and protein synthesis within the first 24 h, these changes being still reversible by androgen administration. Some genes that are associated with cellular proliferation are upregulated (c-myc, H-ras, transglutaminase) as are other more familiar as players in the apoptotic pathway of other cell types (TRPM-2, calmodulin)\textsuperscript{64,65}.

The second stage, which is irreversible, commences when cells become committed to apoptosis, indicated by increasing intracellular Ca\textsuperscript{2+} and decompaction of chromatin as histones and polyamines disappear. As Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent endonuclease activity rises DNA is cut into fragments. ICE-like proteases, which act on the nucleus, and transglutaminase, which cross-links membrane proteins, are activated. The cell membrane forms blebs and breaks into apoptotic bodies which are subsequently phagocytosed. Within 7–10 days after castration the ventral prostate gland of rats is reduced by 80% in mass. The remainder of the cells in the basal epithelium are resistant to androgens, perhaps because they lack the receptor but express bcl-2. Unfortunately, bcl-2 does not neatly explain the evolutionary loss of
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androgen-dependence in tumours, which do not universally express this gene. Further investigation of the molecular basis of this transition is likely to be rewarding.

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