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Tumour rejection antigens: Their role in spontaneous tumour regression

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Exploration of the cancer cell is akin to archaeology: 'We must infer the past from its remnants in the present, and the remnants are often cryptic.

J Michael Bishop: Les Prix Nobel, 1989.

The interaction between the tumour cell and the host immune system has attracted the tumour immunologists for a long time. Immunologists have chosen to concentrate on the surface associated or membrane bound antigens of tumours because of their ability to evoke a strong immune response. Based on their chemical nature, the type of immune response and the nature of the carcinogenic agent, tumour antigens have been classified into several categories, out of which the viral and the chemically induced antigens have been studied extensively. The focus of this article is on the tumour antigens that elicit tumour resistance by stimulating humoral or cellular immune response in a syngeneic host.

TUMOUR antigens, i.e. molecules which elicit immune response in a tumour-bearing host, have been referred by different terms in the history of immunology. Some of these terms like 'aberrant germs', 'factor responsible for regression' and 'new antigenic potentialities' have reincarnated over decades in various forms, e.g. viral antigens, histocompatibility antigens, oncofetal antigens, differentiation antigens, etc.

The long standing goal of cancer biologists has been to stimulate the immunological rejection of tumours. This goal is based on the hypothesis that a cancer cell which develops from its normal progenitor as a consequense of the transformation event, carries a foreign antigen which could be recognized by the host immune system. The focus of this article is on tumour antigens that elicit tumour resistance by stimulating humoral or cellular responses in a syngeneic host.

The capacity of a tumour-specific transplantation antigen to evoke an immune response is influenced by the interaction between the tumour and the host. Therefore, tumour antigens continue to be the interesting molecules to immunologists, primarily due to the role they play in modulating the immune response evoked[1], as well as the possible direct relationship they might have with molecular mechanisms of malignant transformation itself. Table 1 describes some of the tumour rejection antigens that have the ability to elicit an immune response in syngeneic host and thereby resist the tumour challenges.

The classical definition of tumour-specific antigens implied their presence only on tumours, but not on normal tissues. However, among the various tumour-specific antigens discovered so far, normal cellular proteins which can act as antigen and evoke an immune response in a syngeneic host have attracted considerable attention. More recently, the study of heat shock proteins (hsp's) has converged with immunology, more specifically with

Table 1 Tumour antigens defined by their ability to mount an immune response to tumour challenges

Tumour	Antigen	Reference
Murine sarcoma	Gp37	Steinmann *et al* [81]
Rat hepatoma	p100	Srivastava and Das[82]
Murine B lymphoma	Gp80	Koch *et al* [83]
Human melanoma	Gp90	Real *et al* [84]
SV-40 transformed cells	p90–100 (T-antigen)	Anderson *et al* [85]
CdH mouse sarcoma	Gp70	Zbar *et al* [86]
Mouse sarcoma	Gp96	Srivastava *et al* [27]
Mouse sarcoma	p84/86	Ullrich *et al* [16]
B-16 mouse melanoma	B700	Hearing *et al* [62]
Mouse mastocytoma	p60	DePlaen *et al*, [87]
BALB/c sarcoma	p75/82	DuBois *et al* [88]
MuLV induced leukemia	Gp175	Rogers *et al* [89]
A-MuLV transformed lymphoid cells	Gp95	Machida and Kabat[90]

tumour-specific antigens. We would briefly review here this interesting liaison between these two apparently unrelated classes of proteins. Also, the possible functional implications of this observation will be discussed.

Tumour rejection antigens from virally induced tumours have been extensively studied. In such systems, antigens coded by the viral genome are expressed on the tumour cell surface[2]. Some of the viruses which provide a useful laboratory model for the study of tumour antigens are murine leukaemia virus, simian virus 40, adenovirus and polyoma virus. Antigens coded by these viruses are specific and are found on all tumours induced by these viruses[3]. Therefore these antigens were functionally designated as the tumour-specific transplantation antigens (TSTA). Anti-TSTA response in animals was elicited by immunization with purified antigens. The viral antigen is presented to T-lymphocytes after processing by antigen-presenting cells, along with the MHC molecule, thereby inducing cellular immune response[4].

Why are tumour antigens immunogenic?

The term 'immunogenicity' is employed to describe the capacity of a protein to stimulate antibody production, whereas 'antigenicity' reflects on the ability to interact with antibody. The role of phylogeny (degree of foreignness) in determining the immunogenicity and thereby antigenicity is described in detail. In general, antibodies are formed only against those areas of the protein molecule that differ from that of the homologous protein of the immunized animal. Antigenicity is in effect an expression of the surface structure and may be used as a measure of surface alterations arising either from amino acid substitutions or from allosteric changes[5,6].

Tumour antigens have been of considerable interest to immunologists for the fact that they evoke an immune response within a syngeneic host and do so by virtue of being nonself or foreign in parts. The reasons for the immunogenicity of these proteins can be classified as given below:

(i) Alteration in the normal cell physiology upon transformation results in modification of normal cellular components[7,8]. Changes in the levels of glycosylation upon transformation leads to altered pattern of glycoproteins/glycolipids, increased level of proteases/glycosidases leads to exposure of new antigenic determinants.

(ii) Expression of embryonic or foetal antigens[9]. Antigens which are expressed solely or predominantly during foetal development may indeed be recognized by mature immune system as foreign and therefore qualify as tumour antigens. The other example of proteins of the same class is that of carcinoembryonic antigens.

Enzymes such as alkaline phosphatase or aldolase which have distinct adult versus foetal forms, comprise the class of tumour-specific enzyme markers and under appropriate conditions can act as tumour antigens[10].

(iii) Increased levels of normal cellular protein. Proteins which are present at relatively lower levels in the normal tissues are found in elevated amounts in case of transformed cells. The normal cellular protein p53 is shown to be expressed at high levels in mutated form in a wide variety of transformed cells and is shown to be immunogenic in syngeneic host which is indicated by the presence of anti p53 antibodies. This brings up the possibility of a protein acting as an antigen when present in excess amount than in normal cells[11,12].

(iv) Generation of immunogenic complexes due to multimerization. In case of virally transformed cells, the proteins which are encoded by viral information are complexed with normal cellular proteins as a result of which new epitopes are exposed. Such complexes, therefore, may be immunogenic. Possibility of such an immunogenic complex formation between two cellular proteins which are non-immunogenic by themselves exists though there is not sufficient experimental support to the idea[13].

(v) Tumour cells contain new or rearranged information which is characteristic of the carcinogenic agent and is unique to the tumour cell. Upon transformation, genome of a cell undergoes significant changes such as insertions, deletions, point mutations and chromosomal translocation[14,15]. This may result in production of a protein which is normally absent or lead to production of a chimeric protein. Ability of long terminal repeats which are a part of retroviral genome to influence the expression of normal cellular machinery is well documented. Such aberrant products of deregulation can act as immunogens.

(vi) Antibodies are produced against proteins which are different compared to normal (wild-type) protein at the level of primary structure. Tumour-specific antibodies have been detected in tumour-immunized animals[16], however, the structural basis of immunogenicity is still unresolved.

Role played by tumour antigens

Tumour rejection antigens can induce both humoral as well as cellular response. It is well documented that many experimentally induced tumours express antigens that can mediate tumour rejection in syngeneic animals[17]. Cellular immunity plays the key role in this rejection with both T helper cells and cytolytic T lymphocytes being involved[18]. In addition to the antibody response, there is a large body of evidence which describes the processing of antigen into fragments by antigen presenting cells and presentation of the antigen fragment in the grove of MHC molecules to both CD_4^+ T helper and CD_8^+ cytotoxic T lymphocytes[19,20]. Two major pathways of antigen processing and presentation of T cell recognition have been described involving either MHC class I or MHC class II restricted responses. The activated immune cells thus obtained have been shown to

possess cytotoxic activity against syngeneic tumour cells *in vitro*. Such activated immune cells have been shown to transfer immunity in normal syngeneic animals against the tumour in adoptive transfer of immunity assays[21, 22].

It is now generally accepted that T lymphocytes mediate specific immune responses directed against tumour rejection antigens. It has been possible to obtain highly specific cytolytic T lymphocytes from syngeneic animals that have been used in defining tumour rejection antigens[20]. The failure of many tumours to induce a rejection response in immunization and challenge experiments has been interpreted as evidence that these tumours fail to express foreign antigenic determinants[23]. In addition, tumours may also fail to induce an immune response not only because they lack foreign antigenic determinants but also because they do not elicit T cell costimulatory signals[24].

Transplantation antigens of chemically-transformed sarcomas

Members of this class of antigens remain the most provocative candidates for tumour-specific antigens[25–27]. The experimental strategy to study these antigens consisted of administration of a chemical such as 3-methyl cholanthrene A (MCA) which would induce tumours in animals. Such tumour-bearing animals would survive upon excision of the tumour and also become immune to the subsequent tumour challenge. The immunity was observed to be tumour-specific. Sarcomas and carcinomas of a wide variety were studied and which were induced by a number of chemicals in several model systems including mice, rats, guinea pigs, etc. A surprising finding in this regard was the unique immunogenicity of each tumour which was induced using the same chemical, methyl cholanthrene, in mice of the same inbred strain[28]

The characteristics of these tumour antigens can be summarized as follows:

(i) Irradiated tumour cells can be used to immunize the mice against tumour challenge though prior tumour growth and surgical removal remains the most effective immunization procedure.
(ii) The diversity of transplantation rejection antigens appears to be extensive and in a series of 25 independently derived tumours no crossreactivity was found[28].
(iii) Two or more tumours induced in the same mouse can have individually distinct antigens.
(iv) Different chemicals induce distinct antigens and tumour immunogenicity generally varies with the carcinogen[29–32]
(v) Antigens are generally stably expressed during long passage of tumours *in vivo* and *in vitro*.
(vi) Immunity against the tumour can be transferred to non-immunized mice by lymphocytes from immunized donors as shown by adoptive transfer of immunity or by using lymphocytes and tumour cells in *in vitro* immunoneutralization assays.

Recent studies by Old and his group indicate that the antigenic activity resided in a protein molecule of 96 kDa molecular weight which is a surface glycoprotein (Gp96) (ref. 33). A similar molecule was detected from the cell surface extracts of two transplantable mouse sarcomas of Balb/c origin, Meth A and CMS5s. The activity was purified by lectin chromatography and ion exchange chromatography. Tumour immunity elicited by isolated Meth A and CMS5 Gp96 molecules shows the same specificity as immunity elicited by intact tumour cells. Rabbit antisera against Meth A Gp96 detected a Gp96 component in immunoprecipitates of surface-labelled Meth A cells but not CMS5 or other Balb/c cells. Further analysis indicated that individually distinct antigens of these two tumours are 96 kDa glycoproteins which are antigenically related but are distinct. The amino terminal sequencing data reveal presence of an identical stretch of 14 amino acids. Using anti Gp96 antibodies, several clones corresponding to Gp96 have been isolated from cDNA expression libraries. Based on the sequence data obtained using genomic clones as well as cDNA clones, the picture that is emerging indicates that the Gp96 antigens are encoded by a single gene specifying a single mRNA species[34, 35]. Gp96 has also been shown to be present in normal cells and tissues of animal and human origin[34].

P84/86 antigens is another class of tumour antigens isolated from Meth A sarcoma by column chromatography of cytosol fractions. The antigen fraction consists of two isoforms 84 kDa and 86 kDa present in equimolar amounts. Both the isoforms, although phosphorylated, are unglycosylated and do not bind lectins. A surprising feature of this class of antigens is their cytosolic localization which was detected by immunofluorescence. Immunity elicited by P84/86 was of tumour-specific nature[36].

In order to analyse it further cDNAs encoding P84/86 antigens were isolated, sequenced and mapped[37]. Although, the overall homology between Gp96 and P84/86 is only 49% the three-dimensional structure of both the proteins may turn out to be similar.

The genetic origin of diversity of individually distinct transplantation antigens still remains unclear. Although much studied, polymorphism has defied molecular definition. It has been ascribed to systems similar to those operative in case of immunoglobulins or major histocompatibility antigens although evidence in support of these possibilities is scant and inconsistent.

UV-induced tumours

Ultraviolet-induced tumours have been shown to be highly immunogenic[38]. UV-induced tumour 1591 is rejected by normal mice even when multiple large tumour fragments are transplanted. It was easy to develop tumour-specific

CTL clones recognizing tumour-specific rejection antigens, these clones have been used to dissect the complexity of unique tumour-specific antigen[39]. At least four unique tumour-specific antigens have been demonstrated in 1591 tumour which are lost independently of each other[40]. Similar multiple antigens may occur on the tumour P815 (ref. 41).

Major histocompatibility complex antigens

MHC antigens have been shown to facilitate T-cell immune defence against cancer. Certain melanomas and lymphomas have higher expression of MHC class I antigens which seems to be associated with increased growth and metastasis and resistance to NK-mediated killing of the target[42, 43]. MHC antigens are expressed on all adult tissues at varying levels[44] and efforts are on to assess any abnormal expression of these antigens in malignancy. These studies are important as they provide a link between the experimental observations and clinical relevance. A large amount of literature is available describing the expression of HLA antigens in human malignancy[45, 46]. A decreased expression of MHC antigens has been observed in ductal carcinomas[47], basal cell carcinomas[48] and colorectal carcinomas[49]. Similarly *de novo* expression of class II MHC antigens has been demonstrated in primary and metastatic melanoma[50]. Prolonged survival of the patients was observed with decreased HLA-II compared to HLA-I expression and shorter survival time correlated with higher HLA-I and HLA-II expression[50]. Although there are reports of changes in MHC expression in different human malignancies, there is no generalized change described so far which is specific to the malignant process.

In case of animal tumour models, both quantitative and qualitative variations in class I MHC expression have been observed[51]. Virally-induced tumours lacked expression of both H-2K and H-2D antigens[52], whereas variable levels of MHC expression have been seen in carcinogen-induced tumours[53]. There is also evidence to show expression of a novel class I molecule in 1591 tumour which showed abnormal reactivity of alloantigen-specific antibodies with tumour cells[54]. The relationship of such antigens to the unique, i.e. individually specific antigens remained inconclusive because of the absence of any *in vitro* probes. Thus, there seems to be no consistent pattern of MHC expression on established animal tumour cell lines.

Carbohydrate antigens

In a large number of tumours of different origin, the association between aberrant glycosylation and malignant transformation has been clearly demonstrated[55]. Aberrent glycosylation may be the basis of defective cell–cell and cell–matrix interactions, which may be reflected in the abnormal social behaviour of tumour cells, such as invasiveness, uncontrolled growth and metastatic potential. The phenomenon of altered glycosylation is of crucial importance in understanding the antisocial behaviour of tumour cells, as well as in practical applications in diagnosis and treatment of cancer.

Carbohydrate moiety in tumour antigens is associated with either glycolipids or glycoproteins[56]. This was revealed about five decades ago when the glycosylation pattern between normal and *in vitro* transformed cells were compared. Transformation-dependent changes in gangliosides and neutral glycolipids were clearly demonstrated[57]. Oncogenically transformed cells were shown to have glycolipid antigens that are absent in the progenitor cells[58]. With the availability of monoclonal antibodies, a large number of tumour-associated antigens belonging to the globoside or ganglioside series have been identified from different types of tumours. Alterations have been shown to be in the sugar residues in these antigens.

Glycoproteins comprise a large number of tumour antigens which are expressed in tumours of different origin[57]. Glycoprotein as well as glycolipid antigens are usually shed by tumour cells in the blood and are detectable in serum. Predominant glycoproteins which are associated with tumours belong to the mucin type and do not crossreact with glycoplipids[59]. Antigens are usually associated with lung, colonic, ovarian and breast carcinoma and their antigenicity is sensitive to sialidase treatment. The major aberrent glycosylation present in glycoproteins is the predominance of multiantennary structures which is due to enhanced N-acetylglucose addition to the core structure of N-linked glycopeptides[60,61]

Albumin-like antigens

A recent surprising addition to the list of tumour antigens is that of B-700 which is an albumin-like antigen isolated from murine melanomas[5,62]. Melanomas are highly aggressive tumours with well-documented antigenic nature. B-700 is a melanoma-specific glycoprotein antigen with the molecular weight of 67000 which can function as a tumour-specific transplantation antigen. Purified protein (B-700) can be used to immunize normal syngeneic mice against the tumour which significantly inhibits the growth of the melanomas after subcutaneous challenge. Normal melanocytes produce a protein partially homologous to B-700 termed as C-700. Two-dimensional gel electrophoresis of CNBr fragments of B-700 and C-700 indicates that B-700 is a multiple deletion variant of C-700. B-700 can also be isolated in immunoaffinity chromatography. B-700 like antigens are also found on melanomas of four different species and hence are termed as candidate 'panmelanoma' antigens. Amino acid analysis, N-terminal sequencing and immunological cross-reactivity suggest that B-700 is an albumin-like protein. Similarly, a tumour

antigen showing cross reactivity with serum albumin has been reported from cultured neuroblastoma cells[63]. Antibodies able to recognize this antigen were detected in the sera of patients with neuroblastoma.

Our studies on a rat histiocytoma, AK-5 (ref. 64), indicate highly immunogenic nature of this tumour. AK-5 is regressed spontaneously in syngeneic animals and regression is mediated by anti-tumour antibody, CD_8^+-natural killer cells[65] and tumour necrosis factor produced by the host macrophages[66]. We have also looked at the surface antigen profile of these tumour cells. Preliminary observations suggest a strong homology between normal serum albumin and purified AK-5 antigen (unpublished observations). The purified antigen can neutralize the anti-AK-5 antiserum raised in normal syngeneic hosts, thereby protecting the AK-5 cells from lysis in an *in vitro* complement-mediated lysis protection assay. The antigen purified from crude AK-5 plasma membranes induces the generation of cytotoxic antibodies against the tumour in syngeneic hosts. Also, immunoprecipitation experiments performed using anti-AK-5 antibody indicate the presence of a 67-kDa protein confirming its tumour-specific nature. This provides us with an instance of a normal secretory protein being expressed on the surface of a transformed cell of unrelated origin. Albumin is heat shock inducible in the foetal liver[67], and is constitutively expressed in adult life. This unusual stress inducible protein is shown to act as a tumour-specific antigen in mouse melanomas, rat histiocytoma and human neuroblastoma.

Relationship of tumour specific antigens with stress-induced proteins

Heat shock proteins were initially recognized by their increased expression after exposure of cells to elevated temperatures. The phenomenon of expression of heat shock proteins was found to be evolutionarily conserved, i.e., the presence of such proteins was observed from *E. coli* to man. Also these proteins could be grouped into a few distinct families with a high degree of structural conservation. Other stress conditions aiso lead to the induction of heat shock proteins as well. Some of these proteins are expressed constitutively in the absence of any kind of stress in various cell types which is consistent with recently documented evidence where involvement of these proteins as chaperones in protein folding has been described. Except for viral antigens considerable number of tumour antigens show homology with stress-induced proteins[36, 68, 69].

The Gp96 antigens appear to be homologous to HSP100 and chicken HSP108; also GP96 transcripts are inducible upon heat shock[70, 71]. Taken together, both these facts suggest that GP96 class of proteins described previously and p84/86 molecules share significant homology with each other and with heat shock proteins[33]. p84/86 antigens were isolated from Meth A sarcoma using column chromatography and the cDNA encoding p84/86 antigen has recently been isolated and sequenced. The amino-acid sequence as well as the homology at the DNA level indicates that these antigens are murine counterparts of the HSP90 family of proteins[71]

A few rat fibroblast cell lines express a 67,000 dalton protein on the cell surface which can be detected using a monoclonal antibody that recognizes members of the HSP70 family[72]. The remaining clones which do not show expression of this protein can be induced to express p67 under conditions of stress. These studies indicate that HSP70 positive clones are rejected after tumour transplantation while p67 negative clones grow rapidly, although no conclusive evidence exists in support of the immunological function of these antigens.

The homology between the stress-induced proteins and tumour-specific antigens isolated and characterized from various sources is an intriguing observation. This assumes significance in the light of the proposed role for heat shock proteins in antigen processing and presentation. A recent finding in this regard is detection of a protein PBP 72/74 which belongs to HSP 70 protein family and is proposed to play a role in the processing and/or presentation of antigen by binding to processed antigenic peptides and facilitating their interaction with Ia or T-cell receptor. A speculative model indicating possible role of tumour antigens similar to stress proteins has been proposed. The model envisages a comparable role for tumour antigens as that of PBP 72/74 wherein the tumour antigens homologous to stress proteins basically function as carriers of immunogenic peptides[73, 74]. Whether or not the model stands the test of time depends on how successfully it can provide the solution to subsequent questions regarding the specificity of the process. Also, a plausible mechanism needs to be described which would explain the functional association between structurally diverse tumour antigens and a general phenomenon such as antigen presentation. It is suggested that stress-induced proteins may not be tumour antigens *per se* but may be acting as carriers of immunogenic molecules.

Genes coding for tumour antigens

The genetics of tumour antigens is hardly explored. With the help of cloning procedures where gene transfection was carried out, several tum$^-$ variants of mouse tumour p815 were obtained[75]. The transfectants which expressed the antigen were detected by their ability to stimulate the specific cytolytic T lymphocytes[76]. On sequencing, the p815 cell gene was found to be identical to that of the gene isolated from normal DBA/2 kidney cells. The allele borne by the tum$^-$ variants was found to differ from the normal allele by a point mutation located in the coding region[77]. These mutations have been shown to create new antigenic peptides that are presented to the syngenic CTL's by a class I molecule of the MHC.

The gene coding for an epithelial antigen has been cloned from human breast tumour tissue[78]. From the cDNAs and deduced amino-acid sequences, a highly hydrophobic 28 amino-acid peptide has been located towards the carboxy terminus which may correspond to a transmembrane region. Similarly, another tumour-associated antigen, L6, has been cloned and is highly expressed on lung, breast, colon and ovarian carcinomas[79]. The L6 antigen is 202 amino-acids long and is related to a number of cell surface proteins that have been implicated in cell growth.

Summary and prospects

The multistep nature of the carcinogenic process and the complexity of genetic lesions detected in cancer so far contribute largely to the difficult management of cancer. Initial studies on tumour-specific antigens were primarily directed towards generating a diagnostic tool and/or therapeutic means. It has become abundantly clear over the years that no therapy targeted against a single molecule such as tumour-specific antigen can provide a useful solution. Attempts to use tumour-antigens for diagnostic purposes have led to generation of monoclonal antibodies against various tumours and such antibodies have been tried for clinical purposes. e.g. α-fetoprotein antibodies are used to diagnose hepatic tumours. Another series of experiments involving lymphokine-activated killer cells (LAK cells) and tumour-specific antigens have indicated the possibility of using such antigens to specifically expand the clones directed against tumour cells. Such LAK cells have been shown to contribute to tumour rejection[80]. Although promising, this approach has a long way to go.

The relationship between the tumour-specific antigens and stress-induced proteins is a new dimension to the problem in recent demonstration of similarity at the sequence level. Despite numerous attempts, the precise relationship between the initial carcinogenic insult and the nature of the tumour-specific antigen has remained unclear. The significance of this homology needs to be established.

Recent investigations in the experimental animals and in humans have led to a large body of information concerning the biochemical and genetic characteristics of tumour antigens. Hopefully, with the help of this vast knowledge and further progress, it should be possible to improve the diagnosis and control of cancer. These studies should also lead to the molecular characterization and identification of the genetic mechanisms responsible for the diversity among tumour antigens.

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RESEARCH ARTICLE

Abscisic acid-responsive proteins induce salinity stress tolerance in finger millet (*Eleusine coracana* Gaertn.) seedlings

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Pretreatment of finger millet (*Eleusine coracana* Gaertn.) seedlings with 200 mM NaCl significantly enhanced the survival of the seedlings at a lethal stress of 400 mM NaCl and subsequent recovery growth when the stress was withdrawn. However, when the pretreatment included 10 μM abscisic acid (ABA) along with 200 mM NaCl, the seedlings could survive lethal stresses of 500 and 600 mM NaCl and recover remarkably upon withdrawal of stress. Pretreatments with ABA and NaCl resulted in the appearance of several new proteins of 18, 23, 31, 45, 48, 54, 66 and 68 kDa. The ABA responsive proteins were heat-stable and their accumulation increased with ABA concentration in the medium. This increase was also associated with an enhanced tolerance of the seedlings to lethal levels of salinity stress. ABA alone however was not effective either in the synthesis of ABA responsive proteins or in imparting tolerance to salinity stress. ^{35}S-methionine incorporation studies indicated the synthesis of 21, 26, 39, 45, 68, 70, 74 kDa proteins of which 21 kDa was the prominent polypeptide synthesized during induction.

Abiotic stresses such as high or low temperatures and salinity induce the synthesis of stress-shock proteins in plants and animals[1–8]. These proteins have been shown to impart tolerance against lethal levels of stresses[9,10]. The phytohormone, abscisic acid (ABA) that accumulates in tissues under abiotic stresses such as desiccation, salt and extreme temperatures[9–11] has been shown to be involved in the synthesis of the stress-shock proteins.

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